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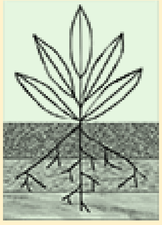

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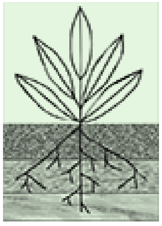

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Humus content and composition of soil from reclaimed Cu post-flotation tailings (Bor, Serbia)

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Abstract

The aim of this work was to determine the content and composition of the humus in the Technosols from reclaimed post-flotation tailings of the Bor copper mine (Serbia). Part of the tailings was reclaimed about 20 years ago by restoring the topsoil with arable soil and planting trees and grasses. Humus parameters were used as indicators for the success of the reclamation. Samples of the natural soil, which served as a borrow pit for the reclamation of the tailing, were analyzed as a control. The humus composition was assessed on the basis of the ratio of humic acids to fulvic acids (Ch/Cf), the ratio of humic acids to total organic carbon (C) (Ch/C) and the ratio of fulvic acids to total organic C (Cf/C). Descriptive statistics, correlation and PCA were used to analyze the data. The decrease in humus content is due to the weak inflow of organic residues into the soil due to the weak development of the vegetation. A significant increase in Cf/C and a decrease in Ch/Cf values are probably the result of the degradation of humus substances under the conditions of a very acidic environment and a lower leaching of fulvic acids. The decrease and degradation of humus in the soil are the result of unfavorable soil quality and unfavorable re-vegetation as a result of inadequate reclamation measures.

Key words: humic acids, fulvic acids, humus degradation, Technosols

Introduction

Mining and related activities cause drastic impacts on terrestrial ecosystems and lead to severe soil degradation (Akala and Lal, 2001). For example, the tailings resulting from the extraction and processing of copper ores damage native vegetation, resulting in large areas of wasteland, and are also source of metal contaminants in local water, air and soil, posing a major threat to biodiversity and human health (Wong, 2003). The Bor copper mining and smelting basin is one of the largest mines in Serbia. Over a period of more than a hundred years, the processing of copper ore has led to the degradation of large areas of land and the formation of tailings. These tailings are also a source of environmental pollution for the surrounding area. In order to restore the function of some of the tailings area, reclamation measures have been carried out on some of them. For example, in 1991, about 16 ha of the post-flotation tailings were reclaimed by restoring the topsoil with natural arable soil and by planting trees and grasses. Thereafter, there was no continuous monitoring or evaluation of the success of the reclamation carried out.

Mineral transformations, stoniness, aggregation and water resistance of structural aggregates, water permeability, accumulation and/or transformation of organic matter, biological activity, etc. are often used as indicators to evaluate and assess the quality of soils on tailings (Asensio et al., 2013). Humus plays an essential role in the functioning of ecosystems as it influences the chemical, but also the physical and biological properties of the soil (Dick et al., 2006). A detailed analysis of humus can provide information on the potential ability of the soil to mobilize or immobilize contaminating substances (such as heavy metals and organic pollutants) (Beyer et al., 2001; Cui et al., 2024; Čokeša et al., 2022; Viventsova et al, 2005), so knowledge of its composition in reclaimed soils is essential for planning follow-up and remediation procedures and for predicting the potential use of the soil (Dick et al., 2006; Viventsova et al., 2005).

The objective of this work was to access quality of soils from reclaimed Cu post-flotation tailings from the Copper mine - Bor (PFT). According to the IUSS Working Group WRB (2007), the investigated soils were classified as Spolic Technosol (Phytotoxic) (Lilić et al., 2014). The aim of this study was to determine the content and composition of the humus as an indicator of the success of the reclamation measures carried out 20 years ago. As a control, the natural soil samples (CNS) from the southern part of the urban area of Bor, which served as a borrow pit for the reconstruction of the tailings, were also analyzed.

Material and Methods

The study area of the PFT dumps of the copper mine is located in the immediate vicinity of the town of Bor in eastern Serbia. The climate in the region is temperate continental and characterized by short, hot summers and long, cold winters. The average annual air temperature is 11ºC, while the minimum and maximum temperatures are -14ºC and 35ºC respectively. The average annual rainfall is 550 mm. Winds from the northwest are the most frequent and strongest, while winds from the east are very variable.

The PFT dump was finally abandoned in 1987. The thickness of the tailings is about 60 meters. Part of the tailings is in a liquid state, another in a muddy state and finally a third (about 25 ha) in a solid state, which was included as such in our investigation. In 1991, an area of about 16 ha was reclaimed by reconstructing the topsoil with arable soil at an average depth of 40 cm. The soil was taken from the southern part of the town, where the residential area has expanded (New Town Centre). Part of the reclaimed area was planted with grass and the other part with trees. Amendment and other measures before and after the reclamation were not carried out. Strong winds dispersed the tailings particles over the reclaimed area (and also over the town and its surroundings). This is one of the reasons for the decay of most of the plants. Today, the tailings site is almost bare, without any vegetation. Birches (*Betula pendula* L.), shrubs (*Rosa canina* L. and *Rubus caesus* L.) from the *Rosaceae* family, and several species from the *Poaceae* family among which *Nardus stricta* L. and *Agropyrum repens* L.

dominate, are only very sporadically present. *Verbascum phlomoides* L. and *Bryopsida spp.* also occur here and there.

As most traces of soil reclamation and possible pedogenetic processes were expected at the soil surface, 21 disturbed samples were taken at a depth of 0-25 cm at a distance of about 30 m in the direction of the letter L (Fig. 1). There is no data on the soil properties of these tailings collected before and/or immediately after remediation. Therefore, surface soil samples (0-25 cm) collected near an area that served as a borrow pit for the remediation of the PFT tailings 20 years ago were used as a control. Five samples were collected in a straight line approximately 100 m apart.

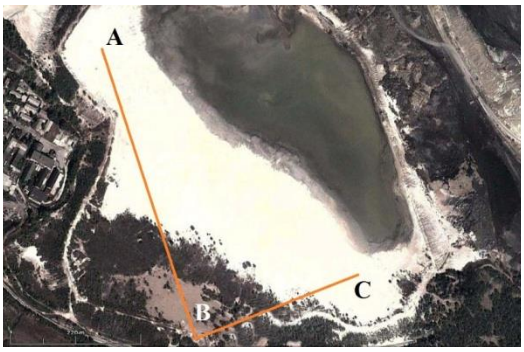

Figure 1. Aerial view of the study area. Direction of sampling: A 44º03´53.59"N, 22º06´51.04"E, 366 m a.s.l.; B 44º03´51.12"N, 22º06´40.12"E, 370 m a.s.l.; C 44º04´06.67"N, 22º06´31.76"E, 366 m a.s.l.

Selected soil properties were determined using the following laboratory methods: Texture by dray saving and pipette method with Na-pyrophosphate preparation, classification of soil texture according to the USDA triangle; total organic C by dichromate oxidation; humus content equals C x 1.72; soil pH in water (soil to water ratio: 1/2.5) by pH measurement; exchangeable acidity by KCl method; cation exchange capacity (CEC) by ammonium acetate method (Reeuwijk, 2002). In addition, the size distribution of water-stable aggregates was determined according to Angers and Mehuys (1993); the stability of soil aggregates based on mean weighted diameter (MWD) was evaluated according to Le Bissonais (1996) and the structural stability index (SI) proposed by Pieri (1992).

The analysis of the humus composition of the soil revealed three humic substances: humic acids (HA), fulvic acids (FA) and humins. HA are not soluble in water in an acidic environment, but become soluble at higher pH values. FA are the fraction that is soluble in aqueous media at all pH values. Humins represent the fraction that is not soluble in an aqueous medium at any pH (or cannot be extracted with an aqueous medium) (MacCarthy, 2001; Piccolo, 2002). The humus composition of the soil was determined according to the method of Kononova and Belchikova (Kononova, 1963). The humus condition was evaluated by the ratio of HA to FAs (Ch/Cf), the degree of humification (index) expressed as the ratio of HA to total organic C (Ch/C) or as the percentage of HA to total organic C

(Ch/Cx100), and as the ratio of FA to total organic C (Cf/C) or as the percentage of FA to total organic C (Cf/Cx100) (Orlov, 1985; Sellami et al., 2008).

Descriptive statistics, correlation analysis (StatSoft, Inc. STATISTICA for Windows, 8) and Principal Component Analysis (PCA) (IBM SPSS Statistics 19) were used to analyze the data.

Results

Reclaimed PFT soil samples are dominantly sandy clay loam, and to a lesser part silt or clay loam (Table 1). Soil structure is characterized mostly by lower content of water-stable large aggregates (>0.25 mm), and, accordingly, high content of water-unstable micro-aggregates (<0.25 mm). MWD values also indicate mostly unstable (48% soil samples), less medium stable (38% soil samples) or stable (14% soil samples) soil structure. SI values, based on humus, silt and clay content, indicate structurally degraded soils (86% soil samples).

Table 1. Physical characteristics of the reclaimed Cu post-flotation tailings and the control soils

Soil properties		Post-flotation tailings				Control natural soils			
		Mean	*Min.*	*Max.*	*Std.dev.*	*Mean*	*Min.*	*Max.*	*Std.dev.*
Coarse sand (%)		23.36	13.37	44.43	7.74	17.13	10.56	22.13	4.16
Fine sand (%)		36.49	26.73	51.78	7.05	27.12	23.71	33.57	3.78
Silt (%)		18.19	7.28	26.76	5.43	27.28	25.56	29.60	1.61
Clay (%)		21.53	5.36	31.04	6.64	28.47	20.20	37.44	6.51
Aggregates in mm (%)	>3	6.24	0.47	17.65	4.26	42.54	23.86	56.20	13.30
	3-2	5.73	0.25	17.28	4.11	9.32	7.41	10.69	1.38
	2-1	16.16	7.04	31.16	7.73	16.04	13.32	21.83	3.43
	1-0.5	18.98	1.83	38.73	7.12	8.03	4.76	11.48	2.68
	0.5-0.25	5.71	1.68	10.51	2.71	1.23	0.65	1.56	0.38
	>0.25	52.68	27.41	75.84	14.53	77.17	67.61	84.42	7.18
	<0.25	47.32	24.16	72.59	14.53	22.83	15.58	32.39	7.18
MWD		0.95	0.44	1.72	0.37	2.91	1.99	3.55	0.65
SI		4.34	1.88	12.97	2.70	10.55	6.94	12.31	2.14

MWD - mean weighted diameter; SI - structural stability index

The reclaimed PFT soil samples are predominantly acidic (slightly to extremely), predominantly very strongly acidic (Table 2). In general, the humus content in reclaimed PFT soils is low. The humus composition is characterized by a mostly low degree of humification (Ch/Cx100 <20% in 87% of the soil samples). The Ch/Cf value is mostly 0.5-1 (62% of the soil samples), less <0.5 (24% of the soil samples) and 1-2 (14% of the soil samples). On non-reclaimed PFT sites, the soil samples contain 0.09±0.07% organic C or 0.16±0.11% humus. The CNS samples generally have a medium humus content, a medium degree of humification and Ch/Cf 0.5-1 or 1-2.

Table 2. Chemical characteristics of the reclaimed Cu post-flotation tailings and the control soils

Soil properties	Post-flotation tailings				Control natural soils			
	Mean	*Min.*	*Max.*	*Std.dev.*	*Mean*	*Min.*	*Max.*	*Std.dev.*
pH in H_2O	5.53	3.84	7.60	1.09	5.74	4.96	6.75	0.83
Exchangeable acidity (cmol$_c$ kg^{-1})	4.42	1.14	6.19	1.55	5.01	3.95	6.02	0.76
CEC (cmol$_c$ kg^{-1})	26.05	10.75	40.00	7.23	43.90	34.00	56.50	9.51
C (%)	0.90	0.38	1.45	0.32	3.41	2.18	4.11	0.75
Humus (%)	1.55	0.65	2.50	0.55	5.88	3.76	7.08	1.28
Ch (%)	0.15	0.06	0.30	0.07	0.66	0.44	0.84	0.18
Ch/Cx100 (%)	16.39	12.00	21.28	2.85	19.42	14.16	22.74	3.17
Ch/C	0.164	0,120	0.213	0.028	0.194	0.142	0.227	0.032
Cf (%)	0.24	0.06	0.45	0.11	0.62	0.47	0.82	0.14
Cf/Cx100 (%)	26.79	11.58	34.74	6.13	18.69	13.58	23.91	4.69
Cf/C	0.268	0.116	0.347	0.061	0.187	0.136	0.239	0.047
Ch/Cf	0.674	0.360	1.820	0.321	1.062	0.860	1.310	0.175

C - total organic carbon; Ch - humic acids; Cf - fulvic acids

Figure 2 shows the results of the PCA obtained from the 19 PFT and 5 CNS surface samples (two samples from the PFT group were discarded as “outliers"). The first two components describe 72 % of the total variance in the dataset (PC1 thus describes 60 %, while PC2 describes 12 % of the total variance within the original dataset), which gives a good picture of the structure of the original dataset in the newly formed PC1-PC2 space. The two groups of samples can be separated along a PC1 axis, i.e. there is a linear segment that allows classification with the lowest possible error. The group cohesion in the PFT is slightly perturbed by the dimensional reduction process, which implies an influence of different parameters on the PFT group structure. Sand, water-unstable micro-aggregates <0.25 mm; Cf/C and exchangeable acidity are the parameters with the highest variance in the PFT sample group, while other parameters (especially the humus content, MWD, HA content and water-stable aggregates >3 mm) can be considered relatively stable. This does not relate to pH in water, clay content and total water-stable macro-aggregates >0.25 mm, as these parameters show greater variance in certain samples from this group than in the rest of the group (which has a direct impact on the cohesion of the group).

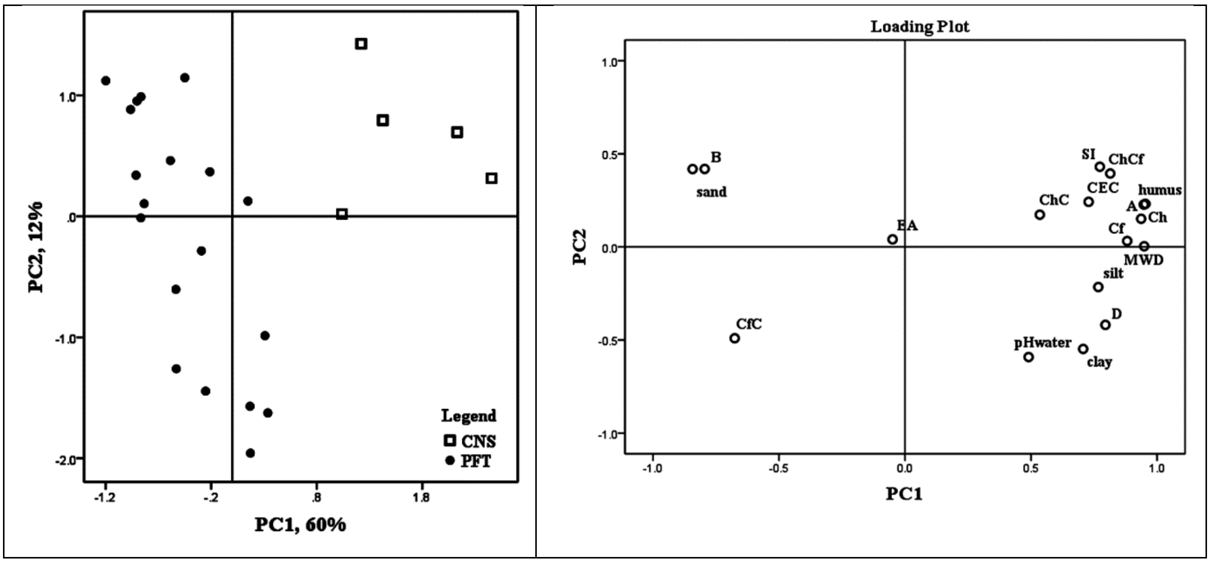

Figure 2. Scores plot (left) of reclaimed PFT (post-flotation tailings) from Copper mine Bor and CNS (control natural soils) samples and loadings plot (right) of humus properties and other chemical and physical parameters (aggregates (mm): water stable A >3, D >0.25; water unstable B <0.25; MWD - mean weighted diameter; SI - structural stability index; EA - exchangeable acidity: Ch - humic acids; Cf - fulvic acids; ChC - ratio of humic acids to total organic C; CfC - ratio of fulvic acids to total organic C: ChCf - ratio of humic acids to fulvic acids)

The humus content was significantly higher in samples that were more silty (Table 3), less sandy and less acidic. The humic acid content was significantly higher when the soil was less acidic and contained more humus. The Ch/C did not correlate significantly with any of the investigated parameters. FA content was higher in samples containing less sand, more clay and silt. The Cf/C was significantly higher in samples containing more clay, less sand and high exchangeable acidity. The soil structure (MWD and SI) is significantly more favorable in samples with more humus and FA.

Discussion

The results of the data examination and analysis thus show clear differences in humus properties and some other soil properties between CNS and PFT, about 20 years after reclamation. In the PFT soils, the humus content is lower and its composition has deteriorated. It is well known that humus depletion is one of the most important processes caused by mechanical or chemical disturbances of the ecosystem (Akala and Lal, 2001; Viventsova et al., 2005). The reclamation of mining sites mitigates the negative environmental impacts associated with mining and leads, among other things, to an increase in the humus content of the soil. In reclaimed mining soils, humus build-up depends on time, climate, technogenic parent material, previous soil properties, vegetation and management before and after reclamation (Akala and Lal, 2001; Colombini et al., 2023; Ivanov and Banov, 2020; Kozłowski et al., 2022; Zhang and Zhang, 2022; Zhao et al., 2020). The authors exclude the influence of the productivity of forests and pastures established on reclaimed mining land and the soil characteristics of the tailings (Akala and Lal, 2001) and the density of plant cover (Dick et al., 2006). More organic matter can be stored on tailings piles that have been reclaimed by restoring the topsoil than on non-rehabilitated tailings (Akala and Lal, 2001). Vegetation is only sporadically present on the reclaimed PFT soils investigated in this study. Remediation procedures included topsoil restoration and planting of trees and grasses, but amendments and other post-reclamation management were neglected, resulting in unsuccessful revegetation. The spontaneous establishment of vegetation is also very weak. The reasons for this are unfavorable general soil properties, which may be due, among other things, to the introduction of mulch by the wind from an unremediated part of the tailings pond. The content of heavy metals is most frequently cited in the literature as the reason for the failure of vegetation and the occurrence of other organisms (Néel et al., 2003; Viventsova et al., 2005). There is also an increased concentration of the microelements As and Cu in PFT soils and very low microbiological activity in the surface layer (Lilić et al., 2014).

Table 3. Correlation coefficient between the humus and some physical and chemical characteristics of the reclaimed Cu post-flotation tailings (**99% confidence level)

Soil properties	Humus	Ch	Ch/C·100	Cf	Cf/C·100	Ch/Cf
Sand	-0.58**	0.45	-0.04	-0.74**	-0.60**	0.54
Silt	0.56**	0.50	0.18	0.63**	0.41	-0.33
Clay	0.53	0.36	-0.10	0.76**	0.67**	-0.61**
MWD	0.60**	0.42	-0.11	0.65**	0.37	-0.31
SI	0.40	0.44	0.36	0.15	-0.35	0.59**
pH in water	0.70**	0.68**	0.38	0.47	-0.06	0.21
Exchangeable acidity	-0.23	-0.33	-0.36	0.18	0.59**	-0.54
CEC	-0.07	0.18	-0.32	0.06	0.32	-0.50
Humus	-	0.94**	0.42	0.86	0.12	0.08

MWD - mean weighted diameter; SI - structural stability index; C - total organic carbon; Ch - humic acids; Cf - fulvic acids

However, there are some contrary experiences, where reclaimed mine soils develop recognizable horizons in a relatively short periods of time and an increased humus content (Akala and Lal, 2001), which is a result of a different mullock quality or/and better reclamation technologies. For example, Ottenhof et al. (2007) reported that the addition of soil organic matter to mine wastes is similar to early stages of soil formation and with time, they expect the formation of well-developed Ah horizon on the surface of mine wastes. Tree vegetation and waste amendment both significantly increased the organic C in the copper mine soils (Spain), but amending with wastes was the only treatment that increased the humified soil organic C concentration to proper values (Asensio et al., 2014). Increase of humified organic C was higher in the amended soils than in the vegetated soils, which led to a higher positive synergy between the organic C supply and microbial biomass development.

The HA and FA content decreased, but the Ch/C values decreased and the Cf/C values increased, so that the Ch/Cf value of the PFT soils decreased. The Ch/Cf value seems to be the most sensitive index for monitoring the humification process. The increase in the value of this humification index, also known as the "degree of polymerization", reflects the formation of complex molecules (HA) from simpler molecules (FA) and a reduction in the non-humic components of the FA fraction (Shen et al., 2014; Sellami et al., 2008). In the reclaimed PFT soils studied, the reverse process probably took place, namely the degradation of complex HA to simpler FA, i.e. humus degradation. However, humic acids extracted from very strongly acidic PFT soils have a higher aromaticity index, degree of oxidation and aromatic condensation than humic acids from slightly acidic and neutral PFT and CNS soils (Radmanović et al., 2020).

Some authors have reported that FA content decreases in polluted soils, e.g. because metals can form soluble complexes with some soil organic substances, such as FA and low molecular weight substances and leach them (Viventsova et al., 2005). In the polluted soils surrounding the "Severonickel" mine, the Ch/Cf was often higher than in unpolluted soils although the humus content in the polluted soils was much lower (Lukina and Nikonov, 1998). Like humus content, its composition may also be related to vegetation, climate and the composition and properties of the soil in which it occurs (Ivanov and Banov, 2020; Swift, 2001).

Compared to the previously cited results, the FA content also decreased, but the Cf/C value increased in the PFT soils. The Cf/C increase can probably be related to some climatic, soil and vegetation factors. One of the reasons could be the lower FA leaching due to the different climatic conditions (less precipitation, 550 mm annual average). FA content and Cf/C as well as clay are significantly positively correlated, which also means that FA leaching is lower due to poor water permeability. In the PFT soils, the total number of bacteria and Azotobacter sp. as well as alkaline phosphomonoesterase (PME) activity showed a positive correlation with clay (Lilić et al., 2014), which can be explained by the high reactivity of microorganisms with the surfaces of clay particles. Thus, higher FA content and Cf/C ratio in clayey soils may be explained by higher microbiological activity. It is likely that FA originate from the decomposition of complex humus acids into simpler forms (Cf/C and clay are negatively correlated, not significant) than from the early stage of humification of organic compounds released from roots or organic litter (due to the very rare vegetation and low inflow of fresh organic residues).

The decrease in humus content followed the decrease in HA (there was a high correlation between Ch and C). Since HA is a nutrient component of humus and its concentration is directly related to the degree of soil fertility (Shen et al., 2014; Barančíkoá et al., 1997), the decrease in HA content indicated a significant decrease in the fertility level of PFT soils 20 years after reclamation. However, the degree of humification (Ch/Cx100 or Ch/C) is lower in the PFT soils compared to the CNS soils, but not so significant. Compared to Ch/C, Ch/Cf better reflects humus degradation by reducing the degree of polymerization and humification.

It is well known that soil organic carbon is a crucial parameter that influences all aspects of physical soil quality (Asensio et al., 2013). A number of studies (e.g. Hayes and Valpp, 1991) have shown that long-term degradation of soil structure is associated with significant losses of soil organic matter. Our results also indicate a deterioration of soil structure; the content of larger water-stable aggregates decreases and MWD values in PFT soils are low compared to CNS. Correlation analyses showed that the humus composition also influences the soil structure. The SI value was higher in samples with a higher Ch/Cf value, which means favorable structural stability in soils with more HA. MWD values were higher in samples with a higher FA content, which is due to a positive correlation between FA and clay, with clay probably having the greatest influence on MWD values.

According to Shen et al. (2014), the rate of HA formation in wastelands composed of mineral copper wastes and tailings (China) was faster than that of FA, and the level of soil fertility gradually increased with vegetation succession in the process of natural restoration. However, the Ch/Cf value was <0.50, the Ch/C value was <0.015 and the soil fertility was still very low even after 40 years of natural restoration. The organic carbon content in the soils of the non-reclaimed PFT areas was on average ten times lower than in the reclaimed area (Lilić et al., 2014). Although the humus of the PFT soils remediated for 20 years was degraded compared to the CNS, the humus properties are still very favorable compared to the above-mentioned 40-year-old mine soils from China. This is proof that these very poor habitats naturally regenerate very slowly and that reclamation is inevitable. The results of this study indicate that reclamation cannot only be partial, but that comprehensive reclamation of the entire area using pre- and post-reclamation measures is necessary.

Conclusion

In PFT soils, about 20 years after reclamation, a decrease in the humus content and a deterioration in its composition can be observed, whereby the content of humic and fulvic acids decreases and the ratio of fulvic acids to humus and of humic to fulvic acids increases. Humus depletion has an effect on the deterioration of other soil properties such as soil structure. Humus degradation is the result of unfavorable soil quality and unfavorable re-vegetation as a result of inadequate reclamation measures. An increase in humus content and an improvement in humus composition would be possible through comprehensive reclamation and improved management before and after reclamation, which would help to avoid the negative effects of overburden from non-reclaimed areas, enable favorable re-vegetation and improve overall soil quality.

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Садржај и састав хумуса у земљишту на рекултивисаним Cu постфлотацијским јаловинама (Бор, Србија)

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Извод

Циљ овог рада је био утврђивање садржаја и састава хумуса у техносолима на рекултивисаним постфлотацијским јаловинама Рудника бакра Бор (Србија). Део јаловине је рекултивисан пре око 20 година реконструкцијом површинског слоја обрадивим земљиштем и озелењавањем дрвећем и травама. Као контрола анализирани су узорци поприродног земљишта које је служило као позајмиште за рекултивацију јаловине. Параметри хумуса коришћени су као показатељи успешности рекултивације. Састав хумуса је процењен на основу односа хуминских и фулвокиселина (Ch/Cf), односа хуминских киселина према укупном органском угљенику (C) (Ch/C) и односа фулвокиселина према укупном органском C (Cf/C). За анализу података коришћена је дескриптивна статистика, корелациона анализа и PCA. До смањења садржаја хумуса долази услед слабог прилива органских остатака у земљиште услед слабог развоја вегетације. Значајно повећање Cf/C и смањење Ch/Cf вредности вероватно су последица разградње хумусних материја у условима веома киселе средине и мањег испирања фулвокиселина. Смањење садржаја и деградација хумуса су последица неповољних особина земљишта и неуспешне ревегетације, а што је резултат неадекватних мера рекултивације.

Кључне речи: хуминске киселине, фулвокиселине, деградација хумуса, техносоли

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Improvement of yield and nutrient composition of orchardgrass in soils with increased nickel concentration by inoculation with *Bacillus* strains

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Abstract

The pollution of soil by potentially toxic trace elements (PTE) is increasing every year and has become a serious problem worldwide. Plant growth is influenced by numerous interactions with the environmental factors and organisms from the surrounding soil, including high concentration of PTE. The aim of this research was to evaluate the possibility of growing orchardgrass in soils with an increased Ni concentration and improvement of the plant quality in these stressful conditions using *Bacillus* inoculums. In addition, the plant growth promoting traits and the germination of inoculated *Dactylis glomerata* seeds with *Bacillus* inoculants was examined. The main goal was to examine the effects of inoculums on plant yield, nutrient composition, and trace elements concentration in orchardgrass biomass. The ability of bacterial isolates to tolerate high concentration of Ni as well as the potential of these bacteria to enhance the germination of orchardgrass seeds was tested in vitro on Petri dishes. A pot experiment was conducted in soil with elevated Ni concentration, and plant dry mass, as well as the content of micro and macro elements in plant material was determined. The results of the pot experiment showed the positive effect of bacterial inoculation on shoot dry weight of orchardgrass, while the nitrogen content in orchardgrass shoots was slightly increased under the influence of bacterial inoculation. Furthermore, the content of microelements in plant material was reduced in inoculated plants.

Keywords: Bacillus inoculants, *Dactylis glomerata*, soil remediation, trace elements, plant quality

Introduction

An essential task in the 21st century is rational and sustainable land use, with the goal of safe food production and biodiversity conservation (Raza et al., 2019). There are estimations that the food demand might increase from 30-35% to 56-62% between 2010 and 2050 to support population growth and eradicate global hunger (van Dijk et al., 2021). Food production will also depend on global climate change that will likely result in degradation and loss of arable agricultural land and more stressful conditions for crop cultivation caused by variations in annual rainfall, temperature fluctuations, heat waves, insect pest attacks, salinity, change in CO_2 and ozone levels, fluctuations of the sea levels (Raza et al., 2019). As this could put global food security at risk, finding effective yet sustainable ways for managing plant stress and improving crop yield, is of great importance for providing food for the growing world population in the changing climate (Kumar et al., 2019). Therefore, finding green and cost-effective alternative is crucial and use of microbial inoculants as bio-fertilizers instead of chemical fertilizers represents a promising and sustainable solution to this problem.

Plant growth under field conditions is influenced by numerous interactions with the environmental factors and organisms from the surrounding soil. Instead of talking about plants individually, a more appropriate term would be a holobiont which takes into account plant and its associated microbiome, also called phytomicrobiome (Backer et al., 2018). Among various rhizobacteria, *Bacillus* spp. are very prominent plant growth-promoting rhizobacteria (PGPR) possessing multiple PGP traits, including nitrogen fixation, mechanisms to increase nutrient availability, phytohormones production, siderophores, antimicrobial compounds, and hydrolytic enzymes, induced systemic resistance (ISR), and tolerance to abiotic stress (Setiawati et al., 2022). Another trait that also makes them good candidates for agricultural and biotechnological application is the ability to produce highly resistant endospores enabling survival under unfavorable environmental conditions, which could also be used to prolong the fertilizer shelf-life. Also, because of their ubiquitous nature and their natural presence in microflora of many plant species, most strains are considered non-phytopathogenic and safe for crops. In fact, numerous studies indicate beneficial effects of various *Bacillus* species on the growth and health of different plant species under normal as well as stressful conditions, including important vegetable crops and grasses (Khan et al., 2020; Masmoudi et al., 2021; Kalam et al., 2020; Lozo et al., 2022; Shin et al., 2021; Kumar et al., 2021; Tahir et al., 2019; Gagné-Bourque et al., 2015; Niu et al., 2016; Rakić et al., 2021) when applied as single inoculant or in the form of bacterial consortium. Also, bio-fertilizers based on different *Bacillus* strains have been registered and commercialized so far.

The pollution of soil by potentially toxic trace elements (PTE) is increasing every year and has become a serious problem worldwide. Sources of heavy metals can be divided into natural and anthropogenic (Alengebawy et al., 2021). Bacteria can affect metal bioavailability in soil and therefore increase or decrease uptake of heavy metals by plants. By secreting various organic acids and biosurfactants, PGPR mobilize metals and make them more available for plant uptake (Yang et al., 2018; Li et al., 2010). Mobilization of metals through biomethylation, forms methylated metal compounds with different solubility and toxicity and leads to metal volatization. PGPR have mechanisms to stabilize metals leading to decreased metal bioavailability, through processes of metal immobilization, precipitation, alkalization and complexation. PGPR can mediate oxidation-reduction processes of metals which result in chemical transformation of metals and change of their mobility which leads to reduced toxicity (Mushtaq et al., 2022). Through processes of biosorption and bioaccumulation, where metal pollutants adhere to cell surface or accumulate inside the bacterial cell, PGPR mediate detoxification of heavy metals and lower the detrimental effects of metals on plants (Wang et al., 2022).

Orchardgrass (*Dactylis glomerata*), also known as cocksfoot, is a perennial species from the *Poaceae* family native to Europe, North Africa, and parts of Asia, but has been introduced worldwide.

It stays productive for 4-8 years and can be harvested 4 times a year. Optimum growth conditions for orchardgrass include annual day temperature from 4.3 to 23.8ºC, and rich soils such as clays and loams with pH ranging from 4.5 to 8.2. It tolerates high temperatures, drought and shade, but it does not endure excessive humidity very well. Due to its good nutritive value and high yields, but also being less competitive and non-toxic compared to tall fescue, it is suitable for planting in mixture with other grasses and legumes, usually with alfalfa and red clover for hay or with white clover for grazing (Aiken et al., 2020, Heuze and Tran, 2015). It has a deep fibrous root and can also be used for soil erosion control, rehabilitation of overgrazed lands or lands disturbed by mining, and stabilization of soil that has been burned (Wang et al., 2023).

There is a small number of studies on how PGPR, and *Bacillus spp.* in particular, affect the growth and nutrient uptake of orchardgrass. Previous work of researchers from this group indicated a potential of different *Bacillus* species to promote growth of orchardgrass, applied as single inoculant or co-inoculated with other species (*Azotobacter* or *Mesorhizobium*) when grown in a single grass culture or as a mixture with legume plants (Stajković-Srbinović et al., 2016, Knežević et al., 2021). There is also limited data on the phytoremediation potential of orchardgrass or how rhizobacterial inoculation influence uptake of nutrients as well as potentially toxic elements. There are studies indicating that orchardgrass is suitable for phytostabilization as it was successfully grown on metal-contaminated, nutrient-poor mine soil with the highest concentration of metals accumulated in the roots (Visconti et al., 2020; Pogrzeba et al., 2019).

The aim of this research was to evaluate the possibility of growing orchardgrass in soils with an increased Ni concentration and improvement of the plant quality in these stressful conditions using *Bacillus* inoculums. In addition, the plant growth promoting traits and the germination of inoculated *D. glomerata* seeds with *Bacillus* inoculants was examined. The main goal was to examine the effects of inoculums on plant yield, nutrient composition, and trace elements concentration in orchardgrass biomass.

Materials and Methods

Sample materials

In this research, two bacterial inoculants were applied: *Bacillus halotolerans* Vig3NK2 and *Bacillus megaterium* DZK1Bh, previously identified by Knežević et al. (2021). Strain Vig3NK2 was isolated from rhizosphere soils of *Medicago sativa* L., while strain DZK1BH was isolated from root nodules of wild *Lotus corniculatus* L. plants. The soil used for the experiment was collected in 2022 from Makiš locality near Belgrade, Serbia.

Tolerance to nickel

Tolerance to nickel for strains was assessed on Nutrient agar (NA) plates (Somasegaran and Hoben, 1994). The NA plates had a range of nickel (II) sulfate heptahydrate of 50 -150 mg L^{-1} (Ni, pH 6). All media were autoclaved at 121°C for 20 min. Bacterial strains were inoculated on prepared media, incubated (28°C, 24 h) and all tests were done in three replications.

Seed germination tests

The ability of two *Bacillus* strains to improve germination of orchardgrass seeds in the presence of different concentrations of nickel was performed *in vitro* on Petri dishes. The experiment was done in a transparent sealed box in a light chamber (22°C, programmed at 16/8 h). An aliquot of 2 mL of overnight culture was added to the microtube with 40 seeds. The mixture was agitated (180 rpm, 5 min) and orchardgrass seeds (inoculated and uninoculated) were placed onto Jensen agar with and without the addition of different concentrations of Ni (0, 50, 100, and 150 mg L^{-1}). The experiment was conducted for 10 days in two independent repetitions and the results were averaged. The results were expressed by the relative seed germination (%) index (RSGI) (Buntić et al., 2017) calculated based on the following equation:

$$\mathrm{RSGI}(\%) = \frac{\mathrm{SG_s}}{\mathrm{SG_c}} \times 100 \qquad (1)$$

where SG_S is germinated seeds in samples, SG_C is germinated seeds in control.

Pot experiment

In each pot 730 g of gravel was used as the bottom layer and 1570 g of soil was added as a top layer. Approximately 0.1 g of *D. glomerata* seeds were inoculated with bacterial inoculums (>10^9 cells mL^{-1}) and placed evenly on the soil in each pot. Treatments used in pot experiment were as follows: T1: (DZK1BH inoculum), T2: (Vig3NK2 inoculum), ØØ: negative control (no fertilizer and no bacterial inoculum added), ØN: treated with recommended dosages of chemical fertilizers (N: 60 kg ha^{-1}, P: 100 kg ha^{-1} and K: 100 kg ha^{-1} and no bacterial inoculum added). Seeds were then covered with a thin layer of soil and pots were kept under greenhouse conditions during one season starting from May 2022. The experiment was set up with three replicates for each treatment and pots were placed in a completely randomized pattern. Plants were harvested before the plant blooming period with two harvests in total, the first in July 2022 and the second in October 2022. After the second harvest, root samples were also collected.

Soil properties

Soil analysis was performed in accordance with SRPS ISO 11464:2004. The basic soil chemical parameters were determined using the following methods: soil pH (SRPS EN ISO 10390:2022); available phosphorus and potassium by the AL method (Egnér et al., 1960); carbonate content (SRPS ISO 10693:2005); and organic matter content according to Kotzmann (Kotzmann, 1930). Concentrations of Ni and other microelements were determined using inductively coupled plasma-atomic emission spectrometry (ICP-AES) (SRPS EN ISO 22036:2024) after the sample was digested with aqua regia according to ISO 54321:2020.

Plant material analysis

Plant and root material was dried at 65°C and grounded in a mill. CNS analyzer (Vario model EL III) was used to measure N (%) in the plant samples (Horneck and Miller, 1998; Miller, 1998). Macro- and micronutrient concentration was determined using THERMO iCAP 6300 Duo ICP-OES (Watson, 1998).

Results and Discussion

Basic soil parameters of soil used for pot experiments are shown in Table 1.

Table 1. Basic chemical properties of soil used for pot experiment

Basic chemical parameters	
pH (KCl)	7.35
pH (H_2O)	8.01
Soil organic matter (SOM) (%)	5.41
P_2O_5 (mg 100 g^{-1})	34.07
K_2O (mg 100 g^{-1})	27.06
$CaCO_3$ (%)	4.03

The soil was alkaline (pH (KCl) > 7.2) with elevated Ni concentrations, which were almost twice higher than the maximum allowed concentrations (MAC = 50 mg kg^{-1}) for this element (Official Gazette of RS, 1994). The content of other trace elements in the soil was lower than MAC. According to the obtained Ni concentration in the soil, the tolerance of applied *Bacillus* strains to the presence of three Ni concentrations in the medium was tested. Both strains, DZK1Bh and Vig3NK2 were able to grow at the concentration of Ni 50 and 100 mg L^{-1}, while bacteria did not grow at the highest Ni concentration of 150 mg L^{-1}.

RSGI gradually decreased with an increase of nickel concentration, in comparison to the control without nickel, which had higher values (Figure 1). Application of both bacterial inoculants increased the percentage of RSGI in all nickel treatments as well as in the control sample. In treatment with nickel concentration of 150 mg g^{-1}, RSGI was two times higher compared to the control sample with the same nickel concentration. Ndeddy Aka and Babalola (2015) showed a similar effect of brassica seeds germination using metal tolerant bacterial strains. Three applied bacterial strains including *Bacillus* (*Pseudomonas aeruginosa* KP717554, *Alcaligenes feacalis* KP717561, and *Bacillus subtilis* KP717559) increased germination index of brassica seeds in the presence of nickel, chromium and cadmium (Ndeddy Aka and Babalola, 2015).

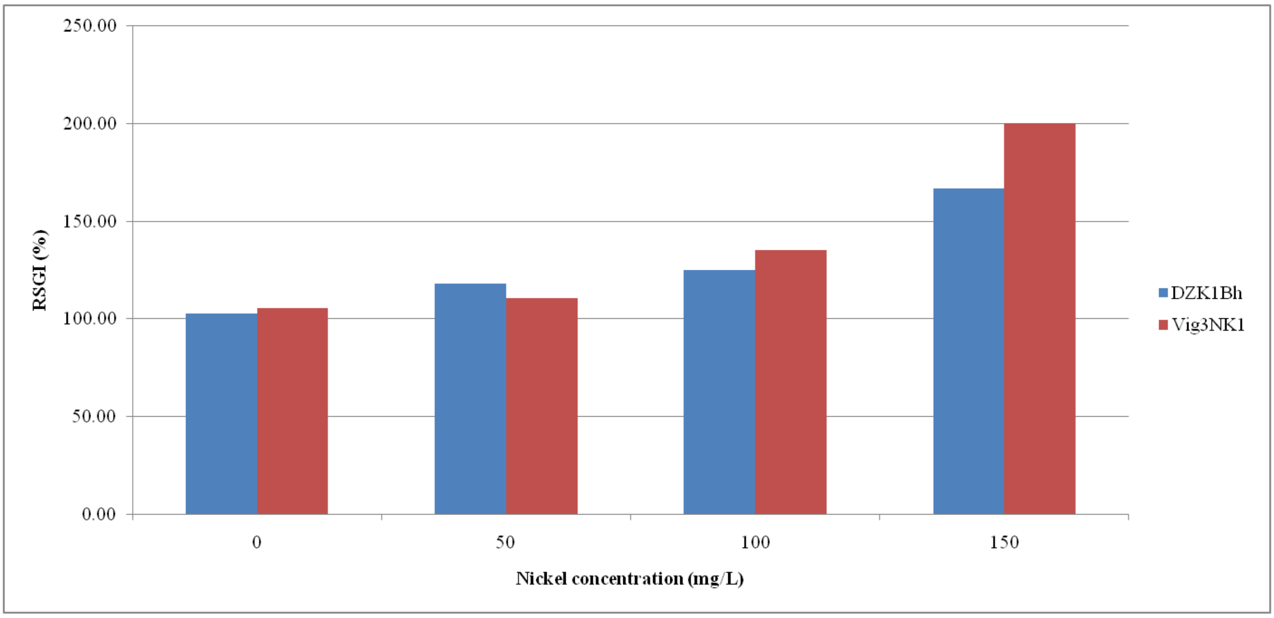

Figure 1. Relative seed germination index of inoculated orchardgrass seeds on different nickel concentrations. Data is presented as average values from three replicates.

The results of the pot experiment showed the positive effect of bacterial inoculation on the shoot dry weight of orchardgrass in both cuttings (Figure 2). Also, the increase in shoot dry weight was recorded for plant roots under the influence of bacterial inoculation. In the first cutting, inoculation by DZK1Bh induced an increase of shoot dry weight 1.61 times in comparison to the uninoculated control. Similarly, inoculation of orchardgrass by the same strain increased shoot dry weight 1.18 times in comparison to the uninoculated control. In addition, the same strain showed the best effect on root dry weight and increased it 1.58 times in comparison to the uninoculated control. Overall, inoculation of orchardgrass by strain DZK1Bh showed the best effect on root and shoot dry weight in both cuttings.

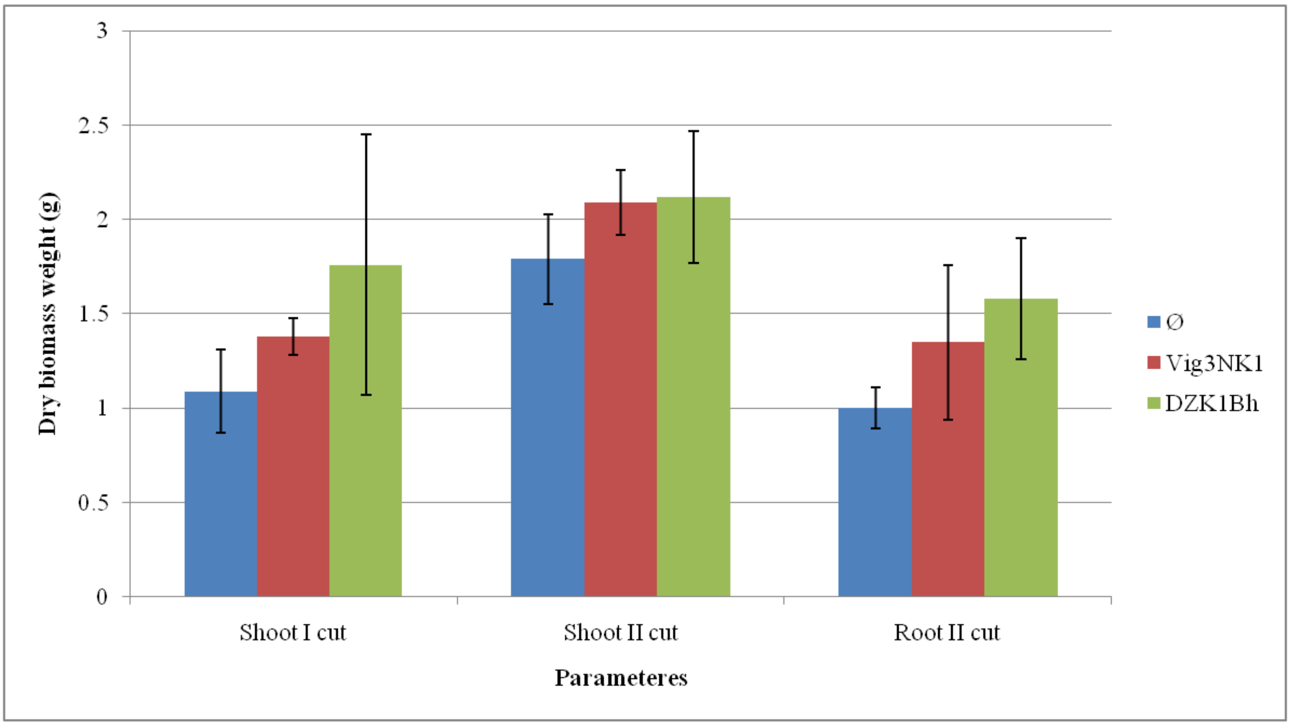

Figure 2. Effect of bacterial inoculation on plant dry weight. Data is presented as average values from three replicates ± standard deviation (SD).

Strain DZK1Bh was previously recommended as an efficient inoculant for improving the growth of orchardgrass in acid soils, where this strain increased the shoot fresh and dry weight (Knežević et al., 2021). Furthermore, the beneficial effects of *B. megaterium* on the growth of different plant species were also previously described by other authors (Bhatt and Maheshwari, 2020; Nascimento et al., 2020; Dahmani et al., 2020).

The results of nitrogen content in the plant material under the influence of bacterial inoculation are shown in Figure 3. Nitrogen content in orchardgrass shoots was slightly increased under the influence of bacterial inoculation in both cuttings. In the first cutting, inoculation by strain DZK1Bh had better effect on the nitrogen content in orchardgrass shoots, while in the second cutting the strain Vig3NK2 showed better results. Regarding the nitrogen content in the root material, values of N% in roots of uninoculated plants had similar values as plants inoculated by strain DZK1Bh. Nitrogen content in plant roots inoculated by Vig3NK2 was slightly lower in comparison to the uninoculated control.

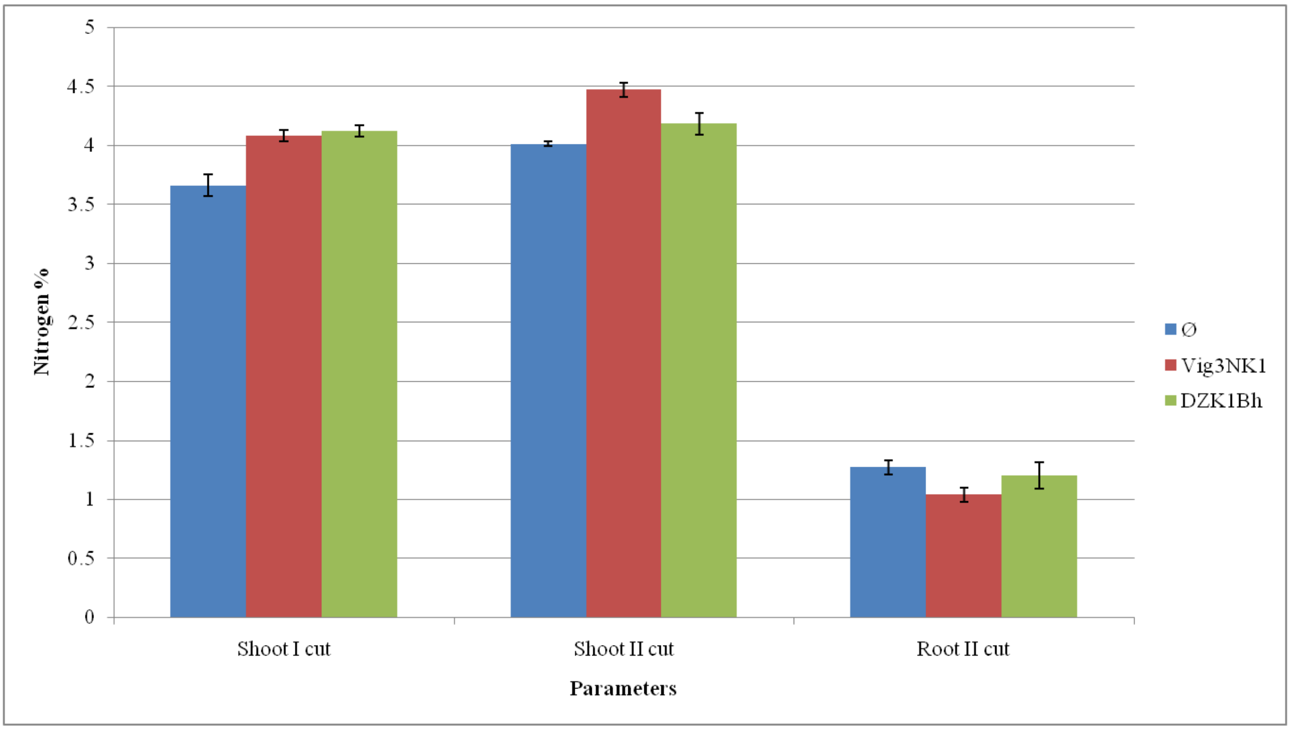

Figure 3. Effect of bacterial inoculation on nitrogen content (N%) in the plant material (shoots and roots). Data is presented as average values from three replicates ± standard deviation (SD).

Overall, the values obtained for N% in the orchardgrass shoot material were higher in comparison to the previously obtained results for the same strain in acid soil, where the highest obtained value for N% was 3.98% (Knežević et al., 2021). Masood et al. (2020) also confirmed the positive effects of inoculation of tomato plant by *B. pumilus*. This could be due to the fact that *Bacillus* species could produce nitrogenase involved in nitrogen fixation and enhance the growth and yield of different plant species (Singh and Shyu, 2024). Processes of atmospheric nitrogen fixation by soil bacteria play important role in supplying soil with nitrogen. Nitrogen fixation can be performed by symbiotic or free-living bacteria. Symbiotic bacteria are associated with legumes, while free-living nitrogen-fixing bacteria are found among different genera. *nifH* gene encoding nitrogenase reductase, among other genetic determinants, is commonly used gene marker for analyzing the ability of bacteria to fix nitrogen (Saxena et al., 2019). In the study by Yousuf et al. (2017), nitrogen fixing ability was observed in different *Bacillus* isolates from tropical estuary and adjacent coastal sea, with *B. megaterium* being most potent. Interesting study about tripartite interaction involving plant, fungus and bacteria concerning N metabolism is done by Paul et al. (2020). Rice inoculated with basidiomycetous fungus *Rhodotorula mucilaginosa* JGTA-S1 which cannot convert nitrate or nitrite to ammonium or fix atmospheric nitrogen, but harbors endobacteria *Pseudomonas stutzeri* with the nitrogen-fixing ability enables the growth of the fungus in N-free conditions and improves growth, nitrogen content and N-use efficiency plants (Paul et al., 2020).

The results of determining the concentration of micro- and macroelements in orchardgrass plants are shown in Tables 3 and 4. In general, concentrations of microelements in both cuts was lower in the plants inoculated by both bacterial isolates, in comparison to the uninoculated control (except in the second cut for Zn and Vig3NK2 inoculation). These results indicate that inoculation of orchardgrass by selected bacterial isolates could reduce the content of microelements in plant material. Similar results regarding the effects of bacterial inoculation on the content of microelements in different species were previously recorded by other authors (Schommer et al., 2023; Wróbel et al., 2023).

Table 3. Concentration of microelements in orchardgrass plants under the influence of bacterial inoculation

Treatment	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**
	mg kg^{-1}									
Ø I cut	BMDL	0.48	0.24	4.61	10.88	447.96	21.10	4.47	0.76	50.77
Ø II cut	BMDL	0.36	0.16	4.00	10.28	304.45	14.00	4.37	0.39	48.33
Vig3NK2 I cut	BMDL	0.42	0.07	3.40	9.27	138.68	20.13	3.74	0.13	46.84
Vig3NK2 II cut	BMDL	0.28	0.11	3.39	10.23	230.74	16.64	3.86	0.28	50.94
DZK1Bh I cut	BMDL	0.38	0.10	3.58	7.36	191.83	20.73	3.62	0.14	39.79
DZK1Bh II cut	BMDL	0.27	0.09	1.75	9.77	204.69	15.35	2.71	0.63	46.15

BMDL – bellow method detection limit

The concentration of Ca in plant material varied depending on bacterial treatment and cutting (Table 4). In plants treated by Vig3NK2 the concentration of Ca was lower in the first cut, and higher in the second, in comparison to the uninoculated control. On the other hand, the concentrations of Ca in plants treated by DZK1Bh were higher in both cuts, in comparison to the uninoculated control. The concentration of K, Mg, and P in plants inoculated by bacterial isolates was higher in comparison to the uninoculated control (except for DZK1Bh treatment in the first cut for K and Mg). Previously it was demonstrated that the effects of bacterial inoculation on the concentration of macro and microelements varies in different cuts in relation to the bacterial treatment, season, location, and plant species (Stajkovic-Srbinovic et al., 2020; Knezevic et al., 2021).

Table 4. Concentration of macroelements in orchardgrass plants under the influence of bacterial inoculation

Treatment	**Ca**	**K**	**Mg**	**P**
		g kg^{-1}		
Ø I cut	9.75	40.14	5.04	2.38
Ø II cut	8.17	33.63	4.77	2.20
Vig3NK2 I cut	8.84	44.45	5.17	2.51
Vig3NK2 II cut	9.52	38.39	4.80	2.63
DZK1Bh I cut	11.36	37.81	4.74	2.22
DZK1Bh II cut	8.59	35.24	4.85	2.30

Bacillus halotolerans Vig3NK2 and *B. megaterium* DZK1Bh have shown good potential as plant growth stimulants under stress conditions caused by increased Ni concentration. They reduced the inhibition percentage of orchardgrass seedling growth by increasing the relative seed germination index. Tested inoculants lowered the concentrations of toxic and potentially toxic metals in orchardgrass biomass and, in general, improved macroelements content in plants cultivated in the pot experiment. The results of this study may help in further field experiments with the phytoremediation of nickel-contaminated soils and production of microbial biofertilizers for contaminated soils.

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Poboljšanje prinosa i nutritivnog sastava ježevice u zemljištima sa povećanom koncentracijom nikla inokulacijom *Bacillus* sojevima

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Izvod

Zagađenje zemljišta potencijalno toksičnim elementima u tragovima (PTE) raste iz godine u godinu i postaje ozbiljan problem širom sveta. Rast biljaka je pod uticajem brojnih interakcija sa faktorima sredine i organizmima iz okolnog zemljišta, uključujući i visoke koncentracije TE. Cilj ovog istraživanja bio je procena mogućnosti gajenja ježevice na zemljištima sa povećanom koncentracijom Ni i poboljšanje kvaliteta biljaka u ovim stresnim uslovima primenom *Bacillus* inokulanata. Dodatno, osobine koje promovišu rast, kao i klijavost semena *Dactylis glomerata* inokulisanih *Bacillus* inokulantima su ispitani. Glavni cilj bio je ispitivanje efekata inokulacije na prinos biomase, sastav nutrijenata i koncentraciju mikroelemenata u biomasi ježevice. Sposobnost bakterijskih izolata da tolerišu visoke koncentracije Ni kao i potencijal ovih bakterija da promovišu klijavost semena ježevice testirano je in vitro u Petri šoljama. Ogled u saksijama sproveden je sa zemljištem koje sadrži povišenu koncentraciju Ni i izmerene su suva masa kao i sadržaj mikro i makro elemenata u biljnom materijalu. Rezultati ogleda u saksijama pokazali su pozitivan efekat bakterijske inokulacije na suvu nadzemnu masu ježevice, dok je sadžaj azota u nadzemnom delu bio blago povišen pod uticajem bakterijske inokulacije. Osim toga, sadržaj mikroelemenata u biljnom materijalu bio je redukovan kod inokulisanih biljaka.

Ključne reči: *Bacillus* inokulanti, *Dactylis glomerata,* remedijacija zemljišta, elementi prisutni u tragovima, kvalitet biljne mase

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Povećanje prinosa soje primenom vodenih ekstrakata biljnog materijala

Increase of soybean yield using aqueous extracts from plant material

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Извод

Abstract

Primena đubriva uslov je ostvarivanju visoke i stabilne, ekonomski opravdane proizvodnje soje. Folijarna primena vodenih ekstrakata biljnog materijala ispoljila je pozitivan uticaj na prinos soje u obe posmatrane godine. Najmanji prinos soje dobijen je na kontrolnoj varijanti, što je statistički veoma značajno manje u odnosu na ostale varijante folijarne prihrane. Folijarna primena vodenog ekstrakta kore banane povećala je prinos soje za 23,46%, folijarna primena vodenog ekstrakta ploda banane povećala je prinos soje za 21,06%, a folijarna primena vodenog ekstrakta koprive za 18,96%. U odnosu na varijantu ogleda tretiranu vodom folijarna primena vodenog ekstrakta kore banane povećala je prinos soje za 16,80%, folijarna primena vodenog ekstrakta ploda banane povećala je prinos soje za 14,54%, a folijarna primena vodenog ekstrakta koprive za 12,55%.

Ključne reči: vodeni ekstrakti, prinos, soja, folijarna primena

Uvod

Introduction

Soja je proteinsko uljana biljna vrsta koja za ostvarenje visokih prinosa zahteva duboka i plodna zemljišta, dobro obezbeđena makro i mikroelementima, a pošto je poreklom iz severoistočne Kine, odnosno suptropskih predela sa povećanom vlažnosti zemljišta i vazduha, za visoke i stabilne prinose zahteva i dovoljnu količinu, kao i pravilan raspored padavina. Unošenje makro i mikroelemenata u zemljište sa osnovnom obradom, na osnovu analize zemljišta i poznavanja potreba biljaka za hranivima osnovni je uslov za ostvarenje visokih prinosa poljoprivrednih proizvoda (Đukić i sar., 2019). U cilju povećanja prinosa i kvaliteta proizvoda sve više se primenjuju folijarni tretmani pošto su folijarna đubriva bogata različitim hranivima i fiziološki aktivnim materijama, sadrže lako usvojive elemente, a efikasnost folijarnih đubriva zavisi od količine hraniva u zemljištu i potreba biljaka za pojedinim elementima, kao i od stanja useva i vremena primene (Miladinov i sar., 2018). Folijarna prihrana soje u fazi intenzivnog porasta povećava prinos (Miladinov i sar., 2018; Ranđelović i sar., 2018), naročito u nepovoljnim godinama, sa izraženim sušnim periodom, ali i u povoljnim godinama za proizvodnju (Dozet i sar., 2013; Dozet i sar., 2015; Ranđelović i sar., 2019). Vodeni ekstrakti biljnog materijala sve se više koriste u proizvodnji različitih vrsta biljaka: u cvećarstvu, povrtarstvu, ali i u ratarstvu, kako u organskoj, tako i u konvencionalnoj proizvodnji (Đukić i sar., 2021). Vodeni ekstrakti biljnog materijala pored makro i mikroelemenata poseduju i fiziološki aktivne materije koje podstiču rast i razvoj biljaka, često imaju fungicidno i/ili insekticidno dejstvo, lako se pripremaju na gazdinstvu, ne iziskuju velika ulaganja i pogodni su za organsku proizvodnju, pošto njihova primena nema negativno dejstvo na životnu sredinu (Mamlić i sar., 2022). Folijarna primena vodenih ekstrakata biljnog porekla povećava prinos mahunarki i ima pozitivan uticaj na kvalitet zrna (Mamlić i sar., 2023) i veoma pozitivan uticaj na prinos soje (Dozet i sar., 2023), pri čemu upotreba vodenih biljnih ekstrakata smanjuje zagađenje zemljišta, vazduha i životne sredine uz dobijanje zdravstveno bezbedne hrane, bez smanjenja visine i kvaliteta prinosa (Dozet i sar., 2017). Primena vodenog ekstrakta ploda banane povećava prinos soje i sadržaj proteina, a smanjuje sadržaj ulja u zrnu, međutim, zahvaljujući povećanju prinosa povećava prinos proteina i ulja po jedinici površine (Mamlić i sar., 2022). Primena vodenih ekstrakata ploda banane, koprive i gaveza, koprive i kore banane povećava prinos zrna soje (Mamlić i sar., 2022a). Prinos i kvalitet soje veoma varira zavisno od klimatskih i zemljišnih uslova u pojedinim godinama i pojedinim regionima gajenja soje (Miladinović i sar., 2013).

Cilj rada bio je da se utvrdi efakat folijarne primene vodenih ekstrakata biljnog porekla na prinos soje na dobro obezbeđenom zemljištu u pogledu hraniva u različitim godinama. Planirana istraživanja doprineće utvrđivanju mogućnosti primene vodenih ekstrakata u konvencionalnoj i/ili organskoj proizvodnji soje.

Materijal i metod rada
Materials and Methods

Dvogodišnji ogled sa folijarnim tretmanima izveden je na eksperimentalnim parcelama Instituta za ratarstvo i povrtarstvo na Rimskim Šančevima, sa srednjestasnom sortrom soje NS Apolo. Varijante ogleda bile su sledeće: Kontrolna varijanta ogleda bez folijarnih tretmana, kontrolna varijanta na kojoj su biljke soje tretirane običnom vodom, varijanta sa primenom vodenog ekstrakta koprive, varijanta sa folijarnom primenom vodenog ekstrakta kore banane i varijanta sa folijarnom primenom vodenog ekstrakta ploda banane. Zemljište za izvođenje ogleda je bilo dobro obezbeđeno fosforom, kalijumom i srednje obezbeđeno azotom, a prilikom jesenje osnovne obrade izostavljeno je osnovno đubrenje. U prolećnom periodu sa predsetvenom pripremom u zemljište je unešeno azotno đubrivo AN (33% N) u količini 100 kgha^{-1}. Ogled je izveden u tri ponavljanja, a veličina osnovne parcelice iznosila je 10m^2 (četiri reda soje sa međurednim razmakom od 50 cm i pet m dužine). Svi folijarni tretmani vršeni su u fazi intenzivnog porasta biljaka, pre cvetanja soje, sa količinom od 300 litara tečnosti po hektaru u kojoj je razređen vodeni ekstrakt u razmeri 1:15. Vodeni ekstrakti pripremani su na sledeći način: 100 grama usitnjenog biljnog materijala preliveno je sa jednom litrom kišnice i uz svakodnevno mešanje sačekan je završetak fermentacije, nakon čega je vodeni ekstrakt proceđen kroz gazu i pre folijarne upotrebe razređen sa vodom u razmeri 1:15. Za dobijanje vodenog ekstrakta koprive korišćen je nadzemni deo (herba) biljke koprive, za vodeni ekstrakt kore banane kora prezrelog ploda banane, a za vodeni ekstrakt ploda banane zreo plod banane zajedno sa korom. Tokom vegetacionog perioda primenjene su standardne agrotehničke mere za proizvodnju soje. U fazi tehnološke zrelosti izvršena je žetva, izmerena masa i vlaga zrna i obračunat prinos po hektaru sa 14% vlage. Rezultati istraživanja obrađeni su analizom varijanse dvofaktorijalnog ogleda, a značajnost razlika testirana je LSD testom na nivou značajnosti 1% i 5% (statistički program „Statistica 13.0“). Rezultati istraživanja prikazani su tabelarno.

Meteorološki uslovi u ispitivanim godinama

Ispitivanja su vršena u 2022. godini, sa izraženom sušom tokom vegetacije soje i u 2023. godini, koja je bila povoljna za proizvodnju soje (Tabela 1). Svedoci smo klimatskih promena u vidu povećanja temperatura, dok padavine pokazuju sve veće oscilacije u pojedinim godinama i smenu kišnih i ekstremno sušnih godina (Đukić i sar., 2018). Prosečne temperature u vegetacionom periodu 2022. i 2023. godine (20,1°C i 20,0°C) bile su iznad višegodišnjeg proseka (18,2°C). U 2022. godini temperature znatno iznad višegodišnjeg proseka zabeležene su u maju (19,2°C), junu (24,0°C), julu (25,1°C) i avgustu (24,6°C), dok su temperature u aprilu i septembru bile ispod višegodišnjih vrednosti (10,9°C i 16,8°C). U 2023. godini više temperature u odnosu na višegodišnje prosečne vrednosti zabeležene su u maju (17,4°C), junu (21,2°C), julu (24,8°C), avgustu (23,7°C) i septembru (21,8°C), dok su srednje dnevne temperature u aprilu bile ispod prosečnih vrednosti (10,8°C). Prosečne temperature u aprilu u obe posmatrane godine bile su veoma niske u odnosu na zahteve biljaka soje, zbog čega je bilo usporeno nicanje i početni porast mladih biljaka. Padavina je tokom vegetacionog perioda soje u 2022. godini (392,3 lm^{-2}) i 2023. godini (405,0 lm^{-2}) bilo iznad višegodišnjeg proseka (377,8 lm^{-2}). Iako je razlika u vegetacionim padavina između ove dve godine samo 12,2 lm^{-2}, ostvareni prinosi u 2022. godini bili su veoma niski, prvenstveno zbog veoma nepovoljnog rasporeda padavina, odnosno izraženim sušnim periodom od maja do zadnje dekade avgusta, uz znatno više temperature u odnosu na višegodišnje vrednosti. Vremenski uslovi tokom vegetacije imaju veliki uticaj na prinos soje (Miladinov i sar. 2018).

Tabela 1. Vremenski uslovi u ispitivanim godinama

Table 1. Weather conditions in the study years

Mesec Month	Srednje mesečne temperature (°C) Mean monthly temperature (°C)			Padavine (lm^{-2}) Precipitation (lm^{-2})		
	2022	2023	Prosek 1964-2021 Average 1964-2021	2022	2023	Prosek 1964-2021 Average 1964-2021
IV	10,9	10,8	**11,8**	54,5	65,0	**47,3**
V	19,2	17,4	**17,0**	17,9	131,0	**68,6**
VI	24,0	21,2	**20,3**	43,6	45,0	**88,3**
VII	25,1	24,8	**21,9**	13,8	58,0	**66,9**
VIII	24,6	23,7	**21,5**	104,0	40,0	**59,6**
IX	16,8	21,8	**17,1**	159,0	66,0	**47,0**
Prosek/Suma Average/Sum	**20,1**	**20,0**	**18,2**	**392,8**	**405,0**	**377,8**

Hemijska svojstva zemljišta

Pre postavljanja ogleda izvršena je analiza oraničnog sloja zemljišta (0-30 cm) u obe godine istraživanja (tabela 2).

Sadržaj humusa određivan je metodom Tjurina - oksidacijom organske materije, lakopristupačni fosfor spektrofotometrijski, a lakopristupačni kalijum plamenofotometrijski.

Tabela 2. Osnovna agrohemijska svojstva zemljišta pre postavljanja ogleda

Table 2. Basic agrochemical soil properties before conducting the experiment

2022. godine						
pH		$CaCO_3$	Humus	Ukupni N	AL-P_2O_5	AL-K_2O
u KCl	u H2O	%	%	%	mg $100g^{-1}$	mg $100g^{-1}$
7,28	7,89	6,12	2,62	0,190	25,3	28,0
2023. godine						
	pH	$CaCO_3$	Humus	Ukupno N	AL-P_2O_5	AL-K_2O
u KCl	u H_2O	%	%	%	mg $100g^{-1}$	mg $100g^{-1}$
7,21	7,67	4,20	2,53	0,194	18,3	23,7

Prema analizama zemljišta može se konstatovati da je 2022. godine ogled izveden na slabo alkalnom, karbonatnom, slabo humoznom zemljištu, srednje obezbeđenom azotom i visoko obezbeđenom fosforom i kalijumom, dok je 2023. godine ogled postavljen na slabo alkalnom, srednje karbonatnom, slabo humoznom zemljištu, srednje obezbeđenom azotom i optimalno obezbeđenom lakopristupačnim fosforom i kalijumom. Iako je zemljište na kome je postavljen ogled u 2022. godini sa većim sadržajem fosfora, kalijuma i humusa, u navedenoj godini su ostvareni veoma niski prinosi soje, prvenstveno zbog veoma lošeg rasporeda padavina, odnosno veoma izraženog sušnog perioda uz znatno više srednje dnevne temperature u odnosu na 2023. godinu.

Rezultati i diskusija
Results and discussion

Tabela 3. Prosečan prinos zrna soje (kgha^{-1})

Table 3. Average soybean grain yield (kgha^{-1})

Folijarni tretmani (B)		Godina (A)		Prosek (B)
		2022	2023	
Kontrola		1.484	2.694	2.089
Kontrola sa vodom		1.653	2.763	2.208
Vodeni ekstrakt koprive		1.776	3.194	2.485
Vodeni ekstrakt kore banane		1.795	3.363	2.579
Vodeni ekstrakt ploda banane		1.831	3.227	2.529
Prosek (A)		1.708	3.048	
LSD	A	B	A x B	B x A
1%	372,4	172,4	186,2	190,5
5%	274,8	126,1	137,0	142,7

Posmatrajući prinos soje po godinama (tabela 3.), uočavaju se statistički visoko značajne razlike između pojedinih godina (1.708 kgha^{-1} u 2022. godini i 3.048 kgha^{-1} u 2023. godini).

Posmatrajući prosečne prinose soje po varijantama đubrenja uočava se da su najveći prinosi ostvareni kod folijarnog tretmana sa vodenim ekstraktom kore banane (2.579 kg ha^{-1}) i vodenim ekstraktom ploda banane (2.529 kg ha^{-1}) što je statistički veoma značajno veći prinos u odnosu na kontrolnu varijantu ogleda (2.089 kg ha^{-1}) i kontrolnu varijantu tretiranu vodom (2.208 kg ha^{-1}). Statistički visoko značajno veći prinos u odnosu na kontrolnu varijantu ogleda, kao i u odnosu na kontrolnu varijantu tretiranu vodom zabeležen je i kod primene vodenog ekstrakta koprive (2.485 kg ha^{-1}). U odnosu na kontrolnu varijantu ogleda folijarni tretman vodom povećao je prinos zrna soje za 5,69%, folijarni tretman vodenim ekstraktom koprive za 18,96%, folijarni tretman vodenim ekstraktom kore banane za 23,46% i folijarni tretman vodenim ekstraktom ploda banane za 21,06%. Pošto je soja biljna vrsta koja povećanjem prinosa reaguje i na male količine vode primenjene u vidu folijarnog tretiranja, a u cilju sagledavanja uticaja primenjenih preparata isključujući uticaj same vode, vodeni ekstrakti su upoređivani sa kontrolnom varijantom gde su biljke tretirane običnom vodom, odnosno sa istom količinom vode po parcelici kolika je i količina tečnosti primenjena na varijantama sa primenom vodenih ekstrakata. Upoređujući prinose soje na varijantama sa folijarnom primenom vodenih ekstrakata sa kontrolnom varijantom tretiranom vodom uočava se da je folijarna primena vodenog ekstrakta koprive povećala prinos zrna soje za 12,55%, primena vodenog ekstrakta kore banane za

16,80% i primena vodenog ekstrakta ploda banane za 14,54%. Zemljište je glavni izvor hraniva za gajene biljke, ali i folijarna prihrana može pozitivno uticati na prinos i kvalitet poljoprivrednih proizvoda (Đukić i sar., 2019).

Posmatrajući prinose u istim godinama a po različitim varijantama đubrenja uočava se da je u 2022. godini najveći prinos ostvaren na varijanti sa folijarnom primenom vodenog ekstrakta ploda banane (1.831 kg ha^{-1}), što je uz ostvarene prinose na varijantama sa primenom vodenog ekstrakta kore banane (1.795 kg ha^{-1}) i vodenog ekstrakta koprive (1.776 kg ha^{-1}) bio statistički visoko značajno veći prinos u odnosu na kontrolnu varijantu ogleda (1.484 kg ha^{-1}). Statistički značajno veći prinos u odnosu na kontrolnu varijantu tretiranu vodom (1.653 kg ha^{-1}) ostvaren je primenom vodenog ekstrakta ploda banane i vodenog ekstrakta kore banane, kao i na varijanti ogleda koja je tretirana vodom u odnosu na kontrolnu varijantu ogleda. U odnosu na kontrolnu varijantu folijarni tretman vodom povećao je prinos soje za 11,38%, folijarni tretman vodenim ekstraktom koprive za 19,68%, folijarni tretman vodenim ekstraktom kore banane za 20,96% i folijarni tretman vodenim ekstraktom ploda banane za 23,38%. U odnosu na kontrolnu varijantu tretiranu vodom, folijarna primena vodenog ekstrakta koprive povećala je prinos zrna soje za 7,44%, primena vodenog ekstrakta kore banane za 8,59% i primena vodenog ekstrakta ploda banane za 10,77%.

U 2023. godini najveći prinos je ostvaren na varijanti ogleda sa folijarnom primenom vodenog ekstrakta kore banane (3.363 kg ha^{-1}), što je uz ostvarene prinose na varijantama sa folijarnom primenom vodenog ekstrakta ploda banane (3.227 kg ha^{-1}) i vodenog ekstrakta koprive (3.194 kg ha^{-1}) statistički visoko značajno veći prinos u odnosu na kontrolnu varijantu ogleda (2.694 kg ha^{-1}) i kontrolnu varijantu tretiranu vodom (2.763 kg ha^{-1}). U odnosu na kontrolnu varijantu ogleda folijarni tretman vodom povećao je prinos soje za 2,56%, folijarni tretman vodenim ekstraktom koprive za 18,56%, folijarni tretman vodenim ekstraktom kore banane za 24,83% i folijarni tretman vodenim ekstraktom ploda banane za 19,78%. U odnosu na kontrolnu varijantu tretiranu vodom, folijarna primena vodenog ekstrakta koprive povećala je prinos zrna soje za 15,60%, primena vodenog ekstrakta kore banane za 21,72% i primena vodenog ekstrakta ploda banane za 16,79%.

Posmatrajući iste folijarne tretmane u različitim godinama uočavaju se statistički visoko značajno niži prinosi ostvareni u 2022. godini u odnosu na 2023. godinu (1.484 kg ha^{-1} i 2.694 kg ha^{-1} na kontrolnoj varijanti ogleda, 1.653 kg ha^{-1} i 2.763 kg ha^{-1} kod kontrolne varijante tretirane vodom, 1.776 kg ha^{-1} i 3.194 kg ha^{-1} kod primene vodenog ekstrakta koprive, 1.795 kg ha^{-1} i 3.363 kg ha^{-1} kod primene vodenog ekstrakta kore banane i 1.831 kg ha^{-1} i 3.227 kg ha^{-1} kod primene vodenogh ekstrakta ploda banane.

Zaključak

Conclusion

Na osnovu ispitivanja uticaja folijarne primene vodenih ekstrakata koprive, ploda banane i kore banane na prinos soje mogu se izvesti sledeći zaključci; Prinos soje bio je pod velikim uticajem klimatskih faktora, prvenstveno količine i rasporeda padavina u vegetacionom periodu soje. Na dobro obezbeđenom zemljištu u pogledu hraniva primena vodenih ekstrakata koprive, ploda banane i kore banane statistički visoko značajno povećavaju prinos soje. U nepovoljnim godinama, sa izraženom sušom, čak i folijarna primena vode može statistički značajno povećati prinos.

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Increase of soybean yield using aqueous extracts from plant material

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Fertilizer application is requirement for achieving high and stable, economically justified soybean products. Foliar application of aqueous extracts from plant material had a positive effect on soybean yield in both observed years. The lowest soybean yields were on the control variant, which is statistically significantly lower in comparison to other variants of foliar fertilization of soybean. Foliar application of banana peel aqueous extract increased soybean yield by 23.46%, foliar application of banana fruit aqueous extract increased soybean yield by 21.06%, and foliar application of nettle aqueous extract by 18.96%. Compared to the water-treated test variant, foliar application of aqueous extract of banana peel increased soybean yield by 16.80%, foliar application of aqueous extract of banana fruit increased soybean yield by 14.54%, and foliar application of aqueous extract of nettle by 12.55%.

Key words: aqueous extracts, soybean yield, soybean, foliar application

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Application of effective microorganisms to reduce mineral nitrogen for feeding wheat

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ABSTRACT

The key to future agricultural production is an ecological approach in order to obtain health-safe food. In this regard, the application of effective microorganisms (EM) in all production systems is a new technological measure. The goal of the research is to determine how the application of EM and different amounts of nitrogen fertilizer affect the yield parameters of bread wheat plants and soil microbial parameters in the rhizosphere. The research was carried out in the field in the period 2017-2019 (factor A) in Serbia, by growing the bread wheat variety Ratarica, in two different sowing densities (400 and 500 grains per m^2) (factor B). Factor C, fertilization treatments: F1 control = NPK (15:15:15) 400 kg ha^{-1} + 150 kg ha^{-1} UREA (46% N); F2 = F1 + 1 foliar treatment with EM preparation; F3 = NPK + 100 kg ha^{-1} UREA + 2 foliar treatments; F4 = NPK + 50 kg ha^{-1} UREA + 3 foliar treatments. The increase in soil microbial parameters was recorded in the F3 treatment (total number of microorganisms by 5.49% and the number of *Azotobacter* by 9.00%) compared to the control ($p<0.01$). The yield of grains was the highest in the F2 treatment (by 5.08%) compared to the control. It is concluded that in sustainable bread wheat production systems, with the application of EM, the amount of mineral nitrogen in the feed can be reduced by 25-30 kg ha^{-1}.

Key words: wheat; fertilizer; effective microorganisms; soil biogenicity; yield

Introduction

At the beginning of the 21st century, agriculture experienced a major technological transformation with the introduction of green directives. The European Green Directive has a series of requirements in food production aimed at reducing the emission of gases that affect the increase in climate change, as well as standards that define a product with a high biological value. Slogans such as Zero Residues, Zero Waste and Zero Kilometers represent a manifesto of a new food concept. This motivated the scientific and professional public to find new directions in food production by introducing ecologically acceptable inputs that would replace chemical preparations in nutrition and plant protection. It has long been known that the introduction of certain groups of microorganisms that are individually compatible with plant species such as rhizobium and legumes reduces the amount of mineral nitrogen in production.

Wheat has great agrotechnical importance in crop rotation. It has a pronounced associative relationship with nitrogen-fixing soil bacteria of the genus *Azotobacter, Agrobacterium, Azospirillum, Beijerinckia*. Numerous studies show that by using different groups of microorganisms as biostimulators in wheat production, it is possible to increase the root mass Naiman, (2009), yield components, grain nutritional properties (Cvijanović et al. 2008; Hijri and Boi, 2018). There are increasing demands to introduce preparations in sustainable agricultural production systems that are the result of EM biotechnology, which is based on a large group of effective microorganisms that have attracted the attention of the scientific and professional public. The potential of the application of effective microorganisms is great for the stimulation of seeds, seedlings, plants and soil regeneration. Mucus and other substances released by EM attach humus and mineral particles, forming clods of the soil, which improves its structure. Microorganisms also produce bioactive substances useful to plants, such as hormones and growth stimulators. Pszczółkowski et al. (2023) states that EM can produce hormones and growth stimulators that act on the cell division of microorganisms and can change the structure of the rhizosphere microbial community and stimulate plant growth. They influence the increase of plants' resistance to stress and the content of bioactive components in the fruits. EM biotechnology is currently used in 140 countries on most continents because effective microorganisms have been shown to be most suitable for sustainable food production systems (Sumbul et al. 2020; Cvijanović et al. 2022;). Zhang et al. (2022) explain the technological progress of applying EM in unfavorable climatic conditions, because they increase the resistance of plants to stress.

The application of EM biotechnology in the future will have an increasing application in the production of crops that occupy the world's largest areas. The goal of the work was to determine the influence of the application of effective microorganisms on the number of basic parameters of soil biogenicity in the rhizosphere of wheat and the height of the grain yield.

Material and methods

Plant material - The culture used in the research is the wheat variety Ratarica, selected by the Institute of PKB Agroeconomics, Serbia. It belongs to the mid-late winter bread variety, whose main morphological characteristic is the retention of the green color of the top leaf until the beginning of the harvest. Also, it has a great power of budding, and the ear is axisless, whitish in color when ripe. Ratarica is a high-yielding variety with a genetic potential for yield in poor conditions (5 to 11 t ha^{-1}), which implies a pronounced tolerance to lodging, drought, stress conditions and grain stiffness.

Research setting and location - The experimental research was conducted in an open field on the experimental plot of the Institute of PKB Agroeconomics, Serbia (ΨN 440 56'; λE 250 28'). The trial was set up in a randomized design with 4 replications. The total area of the sample was 84 m^2, the area of the elementary plot was 5 m^2 (the total number of plots was 32). Agrotechnical measures, typical for sustainable production, were carried out in optimal terms. The previous crop was corn. Sowing was carried out in optimal terms according to the manual sowing system. The protective insulating belt was wheat of the same variety. Three factors were included in the research: Factor Y - year of research, Factor D - Density of seed sowing and Factor F – Fertilization

Agroclimatic conditions - At the meteorological station at the PKB Agroeconomics Institute, Belgrade, Serbia, data on the sum of precipitation and temperature were monitored and collected for research in production years 2016/2017, 2017/2018 i 2018/2019 (Table 1).

Table 1. Average values of mean monthly air temperatures (°C) and sum of monthly precipitation (mm)

Average values of mean monthly air temperatures (°C)											
Year	Monthly										Average
	X	XI	XII	I	II	III	IV	V	VI	VII	
2017	10,8	6,5	2,8	0,2	7,3	7,8	13,9	16,3	21,5	22,6	10,9
2018	9,6	5,9	-0,6	-5,1	3,3	9,9	11,1	17,2	22,5	23,5	9,73
2019	11,0	6,3	3,3	3,1	1,4	5,2	16,5	19,8	21,1	22,1	10,9
Sum of monthly precipitation (mm)											
Year	Monthly										Average
	X	XI	XII	I	II	III	IV	V	VI	VII	
2017	70,6	50,8	10,8	46,5	46,4	78,8	34,4	74,4	89,2	34,8	536,7
2018	70,7	75,0	4,6	18,6	26,9	22,0	46,2	71,6	106,5	40,9	482,3
2019	57,0	48,1	40,6	39,2	47,2	58,2	29,4	80,1	70,1	66,7	537,3

Density of seed sowing - Examination of the sowing density is an important parameter because in this way a high degree of the genetic potential of the fertility of the variety is achieved in different growing conditions. That is why the experiment was sown in two different seed densities: 400 and 500 grains per m^2.

Fertilization - Fertilization was in 4 variants in the form of basic and supplementary feeding (Table 2). The basic NPK complex fertilizer (15:15:15) was introduced in autumn in quantity 400 kg NPK ha^{-1}. Spring feeding of wheat was with mineral nitrogen UREA 46% N in combination with effective microorganisms in the preparation EM Aktiv (trade name). Microbiological preparation EM Aktiv is a liquid preparation, which consists of effective microorganisms (EM), a mixture of different types of microorganisms of photosynthetic bacteria *(Rhodopseudomonas palustris, Rhodobacter sphaeroides)*, milk bacteria *(Lactobacillus plantarum, L. casei, Streptococcus lactis)*, yeasts *(Saccharomyces cerevisiae, Candida utilis)*, actinomycetes *(Streptomyces albus, Streptomyces griseu)* and fungi *(Aspergillus oryzae и*

Mucor hiemalis). EM Aktiv was applied foliarly in different phenophases of plant development, in an amount of 6 l ha^{-1} with water in a ratio of 1:100.

Table 2. Variants of fertilization on the experimental plot

Fertilization variants	Before sowing NPK (kg ha^{-1})	UREA (46% N)	Microbiological fertilizer	Total nutrients N : P_2O_5 : K_2O
F 1	400	150	Control	129 : 60 : 60
F 2	400	150	Phase of stem extension	129 : 60 : 60
F 3	400	100	Phase of stem extension Phase od flowering	106 : 60 : 60
F 4	400	50	Phase of stem extension Phase od flowering Phase of ripening	83 : 60 : 60

Sampling - The harvest was carried out with a combine adapted for harvesting on smaller plots, and the grain yield was pre-parched at 14% moisture and expressed in t ha^{-1}. In order to determine soil microbial properties, samples of rhizosphere soil were taken in the phenophase of technological maturity . The abundance of tested microbial groups were determineted on appropriate nutritive media by a soil dilution plate method. . The total number of microorganisms, was determineted on soil agar (Pochon and Tardeaux, 1962) at a dilution of 10^{-7}. The abundance of *Azotobaceter* was determined on a nitrogen-free medium (Fyodorov's medium) using the method of 25 fertile drops (Anderson, 1978), from a 10^{-1} dilution. Plates were incubated at the temperature of 28℃. The incubation period for the total number of microorganisms was seven days and for *Azotobacter* 48 hours. After incubation, the colonies were counted and the average number of colony forming units (CFU) was calculated at gram of absolutely dry soil (Wollum, 1982).

Statistical analysis - Data were subjected to analysis of variance and regression. For the qualitative factor, means were compared using three-factor ANOVA and LSD test with 1% and 5% significance. All analyzes and tests were performed using the statistical program DSAASTAT (Perugia, Italy).

Results and discussion

Agrometeorological conditions in 2017 and 2019 were uniform and relatively optimal for wheat development. Average daily temperatures and total precipitation were higher than in 2018. In addition, in 2018, a large amount of precipitation (106.5 mm) fell in June, and high temperatures shortened and hindered the grain pouring process, which had a significant impact on the examined parameters. Considering that the precipitation was torrential, the water lay on the surface, which had a significant impact on the composition of the microbiome in the soil.

Total number of microorganisms - Microorganisms includes bacteria, fungi, algae, protozoa and nematodes, because their metabolism is related to the flow of energy and the circulation of elements in ecosystems. Their dynamics, spatial distribution and variation is dependent on numerous of factors (soil properties, temperature and water, concentration of gases and heavy metals, chemical toxicants, nitrogen and phosphorus content). Also, the abundance depends on the genetic and variability of the plant, which is the result of its compatibility with microorganisms and applied agrotechnical measures. One of the key importance is the influence of plants, which refers to the release of various secretions through the roots into the soil (Vives-Peris et al., 2020). Secretions are rich in sugar and acid molecules that directly affect the number and diversity of the microbial population, because they are attracted to them, and the microorganisms break down those molecules.

The average total number of microorganisms was 210.74×10^7 (Table 3). The favorable relationship between temperature and precipitation in 2017 and 2019 had a positive effect on the microbial community. The highest total number of microorganisms was recorded in 2017 (263.58×10^7). Compared to 2019, it was higher only by 0.73%, because the production conditions were similar. However, compared to 2018, the total number of microorganisms was higher by 146.61%. From the obtained results, it can be concluded that the total number of microorganisms reacts significantly to unfavorble agro-climatic conditions compare the optimal. The obtained results are in agreement with Donn et al. (2015), which confirms that during each vegetative season of growing wheat, there are variations in rhizosphere biogenicity. In all three reserach years, the total number of microorganisms was significantly higer at the sowing density of 500 grains per m^2, compared to the sowing density of 400 grains per m^2 (by 19,67%, on average). The obtained results, showed that sowing at a narrow row spacing , the amount of root exudates increases, which is the trigger for the development of the higher number of bacteria. Ayazet al., (2023) determined that the number of plants and root mass directly affect the amount of secretion released into the environment, which implies from the obtained results that the total number of microorganisms increased in the sowing of a higher number of plants per unit area.

The highest total number of microorganisms was recorded in the F3 treatment (216.67×10^7), which was 5.49% higher than in the control (F1) and 1.04% and 3.94% higher than in the F2 and the F4 treatments, respectively. The obtained results are very significant because with reduced amounts of mineral nitrogen and an increased number of treatments with EM Active, stability in the microbiome can be achieved. The above implies that the amount of mineral nitrogen of about 25-30 kg ha^{-1} can be replaced if a preparation with effective microorganisms is used. The obtained results are compatible with the results of Abduladin et al. (2020) who found that in the soybean rhizosphere after the application of effective microorganisms, the total number of bacteria increased by 44.76% to 54.55%, depending on the amount of applied NPK fertilizer. Also, they point out that the results of the research years showed that the impact of EM preparations was the highest in the unfavorable agro-climatic conditions, which gives an advantage to the use of these preparations.

Table 3. Influence of the tested factors on the total number of microorganisms ($\times 10^7$ CFU of absolutely dry soil)

Year (Y)	Density of seed sowing (D)	Fertilization (F)				x̄ Y x F	x̄ Y
		F1	F2	F3	F4		
2017	400	265.67	208.67	282.67	253.00	252.50	263.58
	500	221.67	319.67	299.67	257.67	274.67	
	x̄ D x F	243.67	264.17	291.17	255.33		
2018	400	132.67	77.67	32.67	55.00	74.50	106.88
	500	157.67	140.33	102.67	156.33	139.25	
	x̄ D x F	145.17	109.00	67.67	105.67		
2019	400	232.33	208.67	282.67	270.67	248.58	261.75
	500	222.33	319.67	299.67	258.00	274.92	
	x̄ D x F	227.33	264.17	291.17	264.33		
x̄ D x F	400	210.22	165.00	199.33	192.89	x̄ D	191.86
	500	200.56	259.89	234.00	224.00		229.61
	x̄ F	205.39	212.44	216.67	208.44		
Average 2017-2019							210.74
LSD	Y**	D**	F*	YxD**	YxF**	DxF**	YxDxF**
p<0.05*	0.73	0.62	0.72	1.07	1.025	1.02	1.77
p<0.01**	0.97	0.93	0.97	1.62	1.68	1.37	2.37

Total number of *Azotobacter* - Bacteria belonging to the genus *Azotobacter* are responsible for the fixation of atmospheric nitrogen, which they reduce to molecules available for plants (Sumbul et al. 2020). According to Gothandapani, Sekar, Padaria (2017) numerous researches on the impact of *Azotobacter* in agriculture is related to their activity and production of plant growth stimulants, which further directly affects the yield quality and quantity.

The average number of Azotobacter was 103.35×10^{-1} (Table 4). In years with a more favorable distribution of precipitation and a better air regime in the soil, the abundance of azotobacter was higher. The highest number was determined in 2019, which is 27.05% higher than in 2017 and 74.22% higher than in 2018. As for the sowing density, it can be observed that at a density of 500 grains m^2, the average number of *Azotobacter* for all three years was higher by 65.28% than at a density of 400 grains m^2.

Table 4. The influence of the tested factors on the number of Azotobacter (×10 CFU g^{-1} of absolutely dry soil)

Year (Y)	Density of seed sowing (D)	Fertilization (F)				$\bar{x}$ Y x F	$\bar{x}$ Y
		F1	F2	F3	F4		
2017	400	78.17	94.67	80.67	58.17	77.92	103.35
	500	127.17	112.67	143.17	132.17	128.79	
	$\bar{x}$ D x F	102.67	103.67	111.92	95.17		
2018	400	71.33	79.67	66.67	73.67	72.83	75.38
	500	89.67	95.67	63.67	62.67	77.92	
	$\bar{x}$ D x F	80.50	87.67	65.17	68.17		
2019	400	85.00	109.67	94.67	42.67	83.00	131.33
	500	164.67	129.67	222.67	201.67	179.67	
	$\bar{x}$ D x F	124.83	119.67	158.67	122.17		
$\bar{x}$ D x F	400	78.17	94.67	80.67	58.17	$\bar{x}$ D	77.92
	500	127.17	112.67	143.17	132.17		128.79
	$\bar{x}$ F	102.67	103.67	111.92	95.17		
Average 2017-2019							103.35
LSD	Y**	D**	F*	YxD*	YxF**	DxF**	YxDxF^{ns}
$p<0.05$*	17.67	11.11	10.50	19.25	38.97	31.82	55.11
$p<0.01$**	23.59	16.84	15.17	29.17	52.26	42.67	73.90

The tested fertilization treatments had an effect on the change in the number of *Azotobacter* at the significance level of $p<0.05$. In the F3 tretment, the number of azotobacter was higher by 9.01% compared to the control, and by 7.95% and 17.60% compared to the F2 and F4 treatment, respectively. Kurrey et al., (2018) investigated the effect of different doses of microbial inoculum in onion production and obtained positive results in increasing the content of phosphorus, organic carbon, nitrogen, potassium and sulfur. The research of Ebrahim et al. (2023) showed that six different biofertilizer treatments (*Azotobacter chroococcum*, *Azospirillum lipoferum*, *Flavobacterium* F-40, *Bacillus megaterium*, *Pseudomonas* *fluorescens* and *Rhizophagus irregularis*) in the production of *Zygophyllum eurypterum* seedlings, had a positive effect on the abundance of all fungi, as well as on the morphological and productive properties of the examined plant. The results of Abdel Latef et al. (2021) showed that the application of *Azotobacter chroococcum* and *Alcaligenes faecalis* in the cultivation of canola plants (*Brassica napus*, L.) led to an increase in the concentration of macro and microelements needed by the plant.

The yield of wheat grains is the result of many physiological changes during the development of plants, which are under the direct influence of many factors. The yield of wheat grains is influenced by the characteristics of the variety, climatic factors, biogenicity of the soil and agrotechnical measures.

For the test period 2017-2019, the average yield of 6.00 t ha^{-1} of wheat grains was achieved (Table 5). In 2017, d grain yield was 5.84 t ha^{-1}, while in 2019 the highest yield of 6.53 t ha^{-1} was recorded. The lowest yield was 5.62 t ha^{-1} in 2018, which is primarily the result of unfavorable agrometeorological conditions, because in conditions of uneven distribution of precipitation, the full effect of the applied preparation is missing. According to Malha et al. (2021) the impact of climate conditions in terms of increased rainfall and high temperatures can negatively affect wheat production. Liu et al. (2016) state that higher amounts of precipitation affect yield reduction by up to 7%, which is correlated with the obtained results. In addition, the low yield in 2018 can be explained as a consequence of the lowest total number of microorganisms and *Azotobacter*. The sowing density had no significant effect on the obtained average grain yields However, at a higher seeding density, the grain yield was higher by 2.53% compared to the grain yield at a lower seeding density.

Based on the obtained results, the highest grain yield (6.20 t ha^{-1}) was achieved on F2 tretment. Compared to treatments F1, F3, and F4 the grain yield was higher by 5.08%, 1.47%, and 7.26% respectively. The obtained results indicate a significant contribution of the application of effective microorganisms with a smaller amount of mineral nitrogen in the wheat crop. The application of EM preparation to the soil in combination with manure or the recommended dose of mineral fertilizer resulted in an increase in mung bean yield (Higa et al., 2003). Also, Bajagić et al. (2023) reported that the application of EM Aktiv preparation increased the the soybean yield by 21.52% compared to the control, on average for two years and two soybean varieties.

Table 5. Influence of examined factors on grain yield (t ha^{-1})

Year (Y)	Density of seed sowing (D)	Fertilization (F)				$\bar{x}$ Y x F	$\bar{x}$ Y
		F1	F2	F3	F4		
2017	400	5.80	6.09	5.99	5.67	5.89	5.84
	500	5.54	6.08	6.10	5.44	5.79	
	$\bar{x}$ D x F	5.67	6.08	6.05	5.56		
2018	400	5.97	5.97	5.21	5.42	5.64	5.62
	500	5.59	6.08	5.25	5.44	5.59	
	$\bar{x}$ D x F	5.78	6.02	5.23	5.43		
2019	400	6.04	6.12	7.14	5.63	6.23	6.53
	500	6.45	6.85	6.97	7.06	6.83	
	$\bar{x}$ D x F	6.25	6.48	7.06	6.34		
$\bar{x}$ D x F	400	5.94	6.06	6.11	5.57	$\bar{x}$ D	5.92
	500	5.86	6.33	6.11	5.98		6.07
	$\bar{x}$ F	5.90	6.20	6.11	5.78		
Average 2017-2019							6.00

LSD	Y*	D^{ns}	F*	YxD^{ns}	YxF*	DxF^{ns}	$YxDxF^{ns}$
$p<0.05$*	0.66	0.43	0.31	0.74	0.54	0.44	0.76
$p<0.01$**	0.88	0.61	0.41	1.06	0.72	0.58	1.01

Conclusion

Based on the obtained results, it can be concluded that in the bread wheat production system of the Ratarica variety, the influence of agro-meteorological conditions, the density of sowing and fertilization with effective microorganisms in combination with different amounts of mineral nitrogen had a very significant impact on the rizosphere microbial community. An increase in the number of basic parameters of soil biogenicity (total number of microorganisms and number of azotobacter) was determined. The highest effect was reached with the amount of mineral nitrogen lower by 25-30 kg ha^{-1} than recommended, and with the application of effective micro-fertilizers twice in the vegetation period. At a density of 500 grains per m^2, higher values of all investigated parameters were determined. In general, by applying preparation with effective microorganisms, stable production of bread grain can be achieved in sustainable production systems. This method of production affects the protection of the basic elements of the environment important for food production.

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Primena efektivnih mikroorganizama u cilju smanjenja mineralnog azota u prihrani pšenice

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IZVOD

Ključ za buduću poljoprivrednu proizvodnju jeste еколошки приступ u cilju dobijanja zdravstveno bezbedne hrane. S tim u vezi primena efektivnih mikroorganizama (EM) u svim sistemima proizvodnje je nova tehnološka mera. Cilj istraživanja je da se utvrdi na koji način primena EM i različite količine azotnog đubriva utiču na parametre prinosa biljaka hlebne pšenice i parametre biogenosti zemljišta u rizosferi biljaka. Istraživanja su bila na terenu u periodu 2017-2019, (faktor A) u Srbiji, gajenjem hlebne sorte pšenice, u dve različite gustine setve (400 i 500 zrna po m^2) (faktor B). Faktor C tretmani đubrenja: F1 kontrola = NPK (15:15:15) 400 kg ha^{-1} + 150 kg ha^{-1} UREA (46% N); F2 = F1 + 1 folijarni tretman sa EM preparatom; F3 = NPK + 100 kg ha^{-1} UREA + 2 folijarna tretmana; F4 = NPK + 50 kg ha^{-1} UREA + 3 folijarna tretmana. Povećanje parametara biogenosti zemljišta bilo je pri tretmanu F3 (ukupan broj mikroorganizama za 5,49% i brojnost *Azotobacter* za 9.00%) u odnosu na kontrolu ($p<0.01$). Prinos zrna bio je najveći pri tertamanu F2, 5.08% veći u odnosu na kontrolu. Zaključuje se da se u održivim sistemima proizvodnje hlebne pšenice uz primenu efektivnih mikroorganizama može smanjiti količina mineralnog azota u prihrani za 25-30 kg ha^{-1}.

Ključne reči: pšenica, đubrenje, efektivni mikroorganizmi, biogenost zemljišta, prinos.

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