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## BIOFERTILIZERS IN THE FUNCTION OF SUSTAINABLE DEVELOPMENT

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### Abstract

*The concern about the conservation of natural resources is present in almost all countries today. In the past, environmental protection has been reduced to the protection of nature only, through the protection of plants and animals in the protected areas. Through the development of both technology and environmental consciousness process of improving the environment in all areas has become accepted. Simultaneously the methods and guidelines for the process have been defined in the world as well as in the EU. Commitment of the Republic of Serbia to join the EU means adopting system oriented documents regarding the protection of the environment, organic food production and “soil health” protection.*

*The research is focused on the implementation of biofertilizers as alternative or supplement of mineral fertilizers in crop production, which would ensure the economical production while maintaining a stable yield and environmental protection in the system of sustainable agriculture.*

*The results of this research show that biofertilizers can be successfully used for the protection of the environment and food production, and as such may encourage rural development.*

**Key words:** *system measures, organic farming, biofertilizers, soil*

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## Introduction

During the last few decades, the environmental protection has become a topical issue, being a multi-, inter- and trans-disciplinary field and including environmental protection, pollution prevention and monitoring. Intensive industrial and technological development in the second half of the 19th century led to the first integrated international activities in the field of protection of the environment and ecosystems. After World War II, in 1948, an international conference was held in Fontainebleau, during which the International Union for the Protection of the Environment (IUCN) was established (Teodorović, 2008). Ecological starting points are mainly interconnected and related to securing the non-renewable resources and conservation of biological diversity. The importance of biodiversity protection has been particularly emphasized by the adoption of the Convention in Rio de Janeiro in 1992. This Convention, with its basic standards and guidelines, has become the basis for adoption of legal regulations for all the Member States of the European Union, as well as those intending to join the EU.

So far, the EU has adopted over 200 individual regulations relating to water, air and land pollution, waste management, protection measures imposed on chemical industry and biotechnology, products' standards, environmental impact assessment and the protection of the sea.

At the EU level the general framework of environmental policy is IPPC (*Integrated Pollution Prevention and Control*), given by 96/61EC Directive adopted by the Council of the European Union upon the proposal of the Commission of the European Communities in 1993, was called. Due to the inputs used to achieve maximum genetic yield potential of cultivated plants, agricultural production has transformed into in agri-industry, thus becoming great polluter of natural resources. In such production the most vulnerable area is soil, as the primary means of producing food and fiber. For these reasons, the International Federation of Organic Movement for the production of IFOAM (*International Federation of Organic Agricultural Movements*) was founded in Rio de Janeiro, aiming to facilitate production and processing of food in ecological production management system that promotes and enhances biodiversity, biochemical cycles and biologically active soil. Based on standards and guidelines issued by the IFOAM, the EU adopted the 2092/91 Directive, as well as Codex Alimentarius, which defines international standards for organic production according to FAO and WHO definitions.

Commitment of joining the European Union that has become a policy of the Republic of Serbia, implies the adoption of the system documents compliant with the EU regulations, regarding the protection of the environment. These were the reasons for the Assembly of the Republic of Serbia to establish laws controlling the discharge of pollutants in all environmental spheres, rational use of fuels and resources, as well as measures to ensure the rehabilitation of sites after the cessation of work activities (as is the case in surface coal mining) in late 2004.

Also, the law on organic production and organic products (Službeni glasnik RS, 62/2006), which defined modes of production, processing, transportation, storage and sales of organic products was adopted in 2006.

In order to prevent soil contamination in the agricultural production in the Republic of Serbia, a Regulation (Sl. Glasnik RS 78/2009) which defined maximum allowable amounts of heavy metals in the soil as well as the maximum amount that can enter the soil through processes of fertilization has been adopted (California Code of Regulations, 2001). It became clear that natural resources are not unlimited and that there is a real need to harmonize food production with rational use of resources, so that pollution of environment and agro-ecosystems are reduced to a minimum. (Lang, 1994).

In Serbia, some forms of organic production take place on about 6000 ha, and there is about 9000 ha in the transition process, adding up to only 0.3% of the arable land. This is obviously insufficient area to produce enough food to feed the nation. In addition to that, the agricultural financial subsidies are by far insufficient. This has encouraged many experts and manufacturers to seek ways and methods that can reduce production costs e.g. the cost of all agricultural products, so that their very products become competitive in the domestic and global food market (Cvijanovic, D. *et al*, 2001, 2007).

One of possible ways is adjusted use of mineral nitrogen fertilizers by means of biofertilizers (microorganisms that have the ability to fix atmospheric nitrogen). This way the soil chemisation as a form of fertilization it being reduced, soil biogenicity is preserved, biological cycles are encouraged, and finally the financial investments in production are reduced.

These requirements have directed the research, aiming to determine the impact of various fertilization systems and methods on some parameters important for the soil preservation.

### **The use of biofertilizers in organic production**

Soil microorganisms are the largest group of soil organisms, very heterogeneous and making soil a complex and dynamic system. The number, activity and diversity of microorganisms is considered a significant indicator of potential and effective fertility of the soil. Therefore, the application of microbial inoculants either as biofertilizers, stimulants or phytopathogen biological control agents in food production meets the concept of sustainable agriculture: yield stability and quality and preserving the ecological balance, which has great influence on both, the food health safety and the economic outcome. Biological nitrogen fixation involves the application of effective microorganisms (which fix atmospheric nitrogen, such as *Rhizobium/Bradyrhizobium*, *Azotobacter*, *Azospirillum*, bacteria from *Bacillus*, *Pseudomonas*, *Nostoc*, *Anabaena* geni and mycorrhizal fungi) as inoculates that increase soil biological activity and quality of crops and vegetables (Milošević & Jarak, 2005, Cvijanović, G., 2006, Mićanović, 1997). Mixture of effective microorganisms does not contain genetically modified species but only those normally live in the soil and form an integral part of the microbial niches. According to Babeva and Zenova (1989) between 160 and 190 kg of nitrogen per ha is fixed in the biosphere by the process of biological fixation. Their use can reduce the amount of mineral fertilizers; can affect the microbial processes

that may indirectly express favorable effects on the biogenicity of soil and economic outcomes. Also, using biofertilizers can increase the amount of organic matter in soil.

The importance of organic matter in soil can be observed from a biological point of view. The content and the condition of organic matter in the soil both depend on the activity of microorganisms that simultaneously participate in two different processes, decomposition of organic matter and synthesis of hummus matter. The potential production soil capacity could not be achieved without the participation of micro-organisms (Cvijanović, G., *et al*, 2008). The method of diazotrophs inoculation is underused, because of the need to perform the strains selection according to the genotypes of the plants.

### **The influence of biofertilizers on basic parameters of soil biogenicity**

Microorganisms act as indicators of all changes that occur in the soil, because their level of participation in total soil metabolic activities is at about 60-90%. Their biodiversity is considered the best indicator of potential and effective soil fertility as well as soil degradation. The total number of microorganisms has long been taken as an indicator of the state of the soil. The numbers of *Azotobacter* are important indicators of changes in the soil. *Azotobacter* is the most common kind of associative nitrogen fixing bacteria living in rhizosphere. They are very sensitive to all changes in their habitat, to which they strongly react by population number changes and enzyme activities, therefore are used as reliable indicators of the soil conditions along with the total number of microorganisms. Soil enzyme activity is also a good indicator of the oxidation reaction in the soil or hummification or dehumification processes of organic matter.

During long-term research (2005-2007) at the location of Zemun Polje, on poorly carbonated soil type, the inoculation of corn seed FAO maturity group 700 with associative biofertilizers was (*Azotobacter chroococcum*, *Azospirillum lipoferum*, *Brijerinckia Derox*, *Klebsiella planticola*, *Vineland Azotobacter*, *Pseudomonas Bacillus Bacillus magaterium subtilis*) carried out, at fertilizing level of 80 kgN.ha<sup>-1</sup>. During the period 2008-2009, on degraded soil after completion of surface coal exploitation at the Lajkovac site, experimental seed inoculation with associative biofertilizers (*Azotobacter chroococcum*, *Azospirillum lipoferum*, *Azotobacter vineland*) was performed with the fertilization at 90 kgN.ha<sup>-1</sup>. The study showed that an increase of basic soil biogenicity parameters at the location of Zemun Polje after inoculation, ranging from 3.51 to 7.94%. The same parameters at Lajkovac site shown far greater increase (101.4-18.38%), which correlates to organic matter content of these two soil types, since greater number of parameters were found in chernozem, when only mineral fertilizers were used.

Soil enzyme activity also increased after the seed inoculation at both locations, somewhat less at Lajkovac site (60µgTPF.g<sup>-1</sup>soil) but with higher percentage increase (328%) than in Zemun Polje (481µgTPF.g<sup>-1</sup>soil, 9% increase), which indicates the increase in the overall biodiversity of soil microorganisms.

Table 1. Effects bacterisation and fertilisers on parameters of biogeny soil in rhizosferes maize

Lokalitet	Fertilisers kgN.ha <sup>-1</sup>	Total number of microorganisms		Number of <i>Azotobacter-a</i>		Dehydrogenase activity	
		10 <sup>7</sup> .g <sup>1</sup> soil	Index level	10 <sup>1</sup> .g <sup>1</sup> soil	Index level	µgTPF.g <sup>-1</sup> soil	Index level
Zemun Polje	80 kgN.ha <sup>-1</sup>	153.80	100	168.21	100	438	100
	80kgN. ha <sup>1</sup> +biofertiliz	159.20	103.51	181.50	107.94	481	109
Lajkovac	90 kgN.ha <sup>-1</sup>	106.29	100	93.30	100	14	100
	90kgN. ha <sup>1</sup> +biofertiliz.	214.04	201.4	110.45	118.3	60	428

The results obtained indicate the compatibility of the selected nitrogen fixing species in the inoculums, because seeds bacterisation improved the growth, reproduction and enzyme activity of diazotrophs introduced, which caused an increase in numbers and enzyme activities of autochthonous microbial communities, and provided a good basis for assessing the productive capacity of soil. Biofertilizers introduced to soil cause changes in microbial communities, which compete for space and energy. Those changes are more pronounced during the extreme hydro-thermal conditions. Under the assumption that agro-meteorological factors would be at their average annual values, these results indicate a possible increase in corn yield.

The impact of the biofertilizers inoculation, show the same effect on corn grain protein content and the applied amount of mineral nitrogen. For its high energy content, corn is most used in animal nutrition both in the world and in Serbia. Although the maize proteins are deficient in two amino acids (lysine and tryptophan), they still make about 20% of the total protein in the mixtures, so the ways of their increase are of great ecological importance.

These results show that the bacterisation caused an increase in total protein content in maize grain. In average, the increase for all fertilization levels was 9,22%, and in the variant with bacterisation total protein content was 9.59%, which is very important, because the protein content in maize grain range from 6 to 12 % (Table 2).

Table 2. Effects of bacterisation on total proteins in maize seeds

Mode bacterisation	Proteins	Fertilizers level kgN.ha <sup>-1</sup>				Average
		0	80	120	160	
Inoculated	%	8,87	9,44	10,07	9,98	9,59
	Index level	110,32	108,00	109,10	109,19	109,22
Un-inoculated	%	8,04	8,74	9,23	9,14	8,78
	Index level	100,00	100,00	100,00	100,00	100,00

	LSD 5%	LSD 1%
Inoculationx **	0.176	0.269
	LSD 5%	LSD 1%
Inoculationx Fertilizers**	0.188	0.274

Apart from possible application of associative nitrogen fixators in the production of non-leguminous plants, the application of symbiotic nitrogen fixators in soybean production is a legally regulated measure. It can be said that the soybean production has taken its deserved place on the sowing areas in Serbia. Since 1991 in Serbia, the area planted with soybeans was about 45 530 ha, showing tendency of growth (Hrustić *et al*, 2002). The largest area under soybean to date was 157 000 ha, during year 2006 (Miladinovic, *et al*, 2008). Soybean not only is the raw material for the growing number of products in human nutrition, but holds an important place in the development of organic agriculture concept. Soy is believed to be an excellent pre-crop, especially for winter wheat, and a good undercrop in the joint sowing (Kovacevic, *et al.*, 2005; Lazic *et al*, 2008).

Sowing soybeans as pre-crop, combined or stubble crop, according to FAO and WHO definition, is the way of production management that promotes the recovery of ecosystems including their biodiversity, biological cycles and methods that largely exclude or correct the use of inputs (Kádár, 2007). Its importance in organic food production comes from the fact that it has low mineral nitrogen requirements, thanks to its genetical determination to live in symbiosis with *Bradyrhizobium japonicum*, bacteria that fix nitrogen from the atmosphere. After they enter the soil, the extent of their propagation and relationships with the present microbes will vary. They stimulate plant growth exercising inhibitory effect on the competitive, pest and parasitic microflora present in spermatosphere and rhizosphere; enter competitive relationships for space and food with other microorganisms from the microbial community, which causes changes in the entire microbial community (Németh, 2006, Milošević, 2008).

*Table 3. Effects of bacterisation and fertilisers on parameters of soil biogenicity in maize rhizosphere*

Locality	Fertilisers kgN.ha <sup>-1</sup>	Total number of microorganisms		Number of <i>Azotobacter-a</i>		Dehydrogenase activity	
		10 <sup>7</sup> .g <sup>1</sup> soil	Index level	10 <sup>1</sup> .g <sup>1</sup> soil	Index level	µgTPF.g <sup>-1</sup> soil	Index level
Zemun Polje	40 kgN.ha <sup>-1</sup>	53.7	100	188.5	100	443	100
	40kgN. ha <sup>1</sup> +biofertiliz	150.2	279.7	278.7	147.8	474	107.1
	60 kgN.ha <sup>-1</sup>	38.6	100	191.7	100	433	100
	60kgN. ha <sup>1</sup> +biofertiliz.	105.1	272.3	292.9	152.8	525	121.2
Lajkovac	90 kgN.ha <sup>-1</sup>	28.6	100	58.4	100	113	100
	90kgN. ha <sup>1</sup> +biofertiliz	90.8	317.5	93.9	212.2	174	153.9

## Conclusion

Based on the results of this research, it can be concluded that in safe food production systems, the inoculation of corn and soybean seeds by compatible species of bacteria that fix nitrogen from the atmosphere can be successfully performed, thus reducing the need for mineral nitrogen in quantities of about 60-90 kg N ha<sup>-1</sup>. This measure should be taken into account with special care, especially in the case of soils contaminated with excessive use of mineral nitrogen fertilizers and the soils that are in the process of biological recultivation. Introducing nitrogen-fixators to the soil stimulates growth of useful autochthonous microbial population whose metabolites participate in the processes of creating and maintaining organic matter in soil as well as in soil phytopathogens control.

Creating associations with roots and using root exudates for their own metabolic processes, they increase their biomass and activity, thus increasing the biogenicity of soil. This intensifies the circulation of the essential elements and therefore makes the necessary supply of plant nutrients better.

This certainly is important in terms of ecology and economy, and therefore provides an opportunity for rural development through agro-tourism and ethno-production of safe food.

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