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THE INFLUENCE OF MICROBIAL BIOFERTILIZERS ON THE BALANCE OF NUTRITIONAL ELEMENTS ON SOILS WITH DIFFERENT DEGREES OF EROSION

Leonid POPOV, Tatiana DAVID

Institute of Pedology, Agrochemistry and Soil Protection "Nicolae Dimo", 100 Ialoveni Street, Chisinau, Republic of Moldova

Corresponding author email: leonidpopov944@gmail.com

Abstract

Currently, the problem of degradation of the effective soil fertility of the Republic of Moldova is reaching a critical scale, being unsatisfactory on 90% of the agricultural land. The annual uncompensated losses of organic matter as a result of mineralization exceed the level of 700 kg/ha, and the total deficit resulting from erosional losses is 1100 kg/year. The main factors that conditioned the establishment of a negative balance of organic matter are the lack of crop rotations, the intensification of erosion processes, the increased costs of chemical fertilizers, as well as the unfavorable climatic conditions manifested by excessive droughts with annual frequency. All together lead to the intensification of chemical, physical and biological degradation processes. In this article, the results of the research are presented, regarding the influence of microbial biofertilizers from the PGPB group (plant growth promoting bacteria) on the balance of nutrients on soils with different degrees of erosion. It was demonstrated that the application of bacteria suspensions, Ps. aureofascens, Az. chroococcum and Ps. fluorescens, contributes to the more intensive accumulation of some nutritional elements, which leads to balancing plant nutrition, as well as increasing the fertility of degraded soils.

Key words: *degradation, erosional perder, organic matter, biofertilizers, fertility.*

INTRODUCTION

Soils are the main natural wealth of every country. The food security of the country, the well-being of the population in both rural and urban areas, as well as the quality of the environment, largely depend on their quality and production capacity. However, the long exploitation of soils without observing the laws of agricultural science and the technologies of cultivation of crops leads to their degradation. In the last 20-25 years, all forms and types of soil degradation have intensified and expanded, especially through erosion, dehumification, drying up of nutrients, salinization and alkalization, destructuring, secondary compaction, reduction of biodiversity, etc. Erosion is the most serious and widespread form of soil degradation. Surface and deep erosion is conditioned by natural and anthropogenic factors (Dobrovolyskij et al., 2008). The natural factors are: the geomorphological conditions (the bumpy relief, especially in the central and southern areas of Moldova), the torrential character of the atmospheric deposits, the low degree of soil coverage with vegetation in the critical erosion season, the low resistance of the soils to erosion. The main anthropogenic actions that led to the acceleration of erosion processes refer to: the excessive capitalization of the land fund (about 74% of the total) with the inclusion in the agricultural circuit of lands with an increased degree of inclination; abandonment of zonal anti-erosion embankments; quota increased grazing crops on sloping land; the lack of drainage regularization strips on the slopes; the lack of the simplest agrotechnical and phytotechnical anti-erosion measures on the land in Soil erosion is a natural process and has an irreversible character (Dimitrov et al., 2004). The fertile soil washed from the slope by erosion is lost practically forever. The speed and result of the manifestation of erosion depend on the natural conditions and human activities. The surface of eroded soils expanded over 44 years (1965-2009) by 284.0 thousand ha, increasing annually by 6.6 thousand ha. According to the Land Cadastre of the Republic of Moldova from 01.01.2009, agricultural lands with different degrees of erosion constitute 878 thousand ha or 35% of the total.

Depending on the degree of erosion, soil fertility decreases from 20% for slightly eroded soils to 60-80% for strongly eroded ones. The damages caused to the national economy by erosion are colossal. Annual soil losses amount to about 26 million tons. This amount of fertile soil contains 700 thousand tons of humus and 84 thousand tons of nitrogen and phosphorus. The cost of agricultural production, lost due to soil erosion, is estimated at 873 million lei. In total, the annual direct and indirect damages as a result of erosion processes amount to 2 billion 723 million lei. Erosional processes also have a negative impact on soil biota. Microorganisms, which facilitate the absorption of nutrients or increase their availability, stimulate plant growth, are commonly called biofertilizers. Biofertilizers are considered as an alternative or complement to chemical fertilization to increase the production of agricultural crops at low cost and to improve the quality of eroded soils. The products (derivatives) of plant growth-stimulating bacteria, so-called PGPB, are most often used. There are some PGPB, the best studied, that can fix nitrogen, solubilize mineral nutrients and mineralize organic compounds. Data from the literature indicate an increased tendency to increase production yield due to the incorporation of nitrogen-fixing PGPB bacteria into the rhizosphere (Martínez-Viveros et al., 2010). Based on microorganisms and metabolites, a series of new biological substances are created, which allow a significant reduction in the amount of chemical substances used in agriculture. Various aspects remain unstudied, especially in the direction of the selection of bacterial strains, taking into account the specifics of growth conditions and plant species, as well as the complex use of microelements with microorganisms in order to increase their efficiency and reduce the level of erosion and environmental pollution.

MATERIALS AND METHODS

The experiments were mounted on moderately eroded clay-loam carbonate chernozem. The total thickness of the humifier profile is 80 cm. The glomerular-bulky structure is characteristic only for the arable layer (0-31 cm). The chernozem of the experimental batch is characterized by a low content of carbonates in the arable layer (2.3%), with depth their amount increases, reaching the maximum in the BC horizon in the 81-95 cm layer (12.8%). The pH_{H_2O} - value is 8.4-8.6 and is stable throughout the profile. The soil of the experimental lot is weak humus (2.67%) in the arable start, but has a deep humus profile, in the state 81-95cm it contains 1.39% humus (Table 1). The sum of exchangeable cations (Ca^{++}, Mg^{++}) is 31.4 me/100 g sol.

Table 1. Physico-chemical characteristics of clay-clay carbonate chernozem

Genetic horizon	The depth, cm	Humus, %	$CaCO_3$, %	pH_{H2O}	Exchangeable cations, me/100 g sol		
					Ca^{++}	Mg^{++}	Sum
A_p	0-31	2.67	2.3	8.4	27.5	3.9	31.4
A	31-46	2.42	3.5	8.5	27.4	3.1	30.5
B^1	46-65	1.62	4.9	8.5	25.7	3.9	29.6
B^2	65-81	1.39	6.8	8.6	21.3	3.5	24.8
BC	81-95	1.39	10.8	8.6	18.2	5.9	24.1
C	95-150						

Vines, Sauvignon variety, young plantation (5-6 years old) served as the object of study. The plants were foliarly treated with bacterial metabolites during the vegetation period five times with an interval of 12-15 days (5 true leaves, 3-4 days before flowering, after flowering, in the phase of intensive plant development, before ripening of the baccalaureate). Based on the preliminary research, the strains of *Pseudomonas aureofascens* and *Ps. fluorescens* were selected. Plants treated with water served as a control. The bacteria were grown on liquid nutrient medium for 24 hours at a temperature of 27˚C with a titer of 10^{10} UFC/ml and applied in the form of metabolites. To obtain the bacterial metabolites, the concentrated suspensions were centrifuged at 8 thousand rpm for 20 minutes in order to precipitate the bacterial cells and obtain the metabolic products. Soil samples for analysis were taken from the experimental lot at two depths - 0-30 and 30-60 cm. The following indices were determined in the soil and plant organs: the content of NPK, total N, NH_4, trace elements Fe, Cu, Mn, Zn, humus, pH, carbonates ($CaCO_3$), exchangeable cations (Ca^{++}, Mg^{++}).

The results were analyzed statistically, using the software - the Statistica programming package on the computer. The data are presented in tables and figures showing the general arithmetic mean of the results from three experiments.

Table 2. Content of total nitrogen, phosphorus and potassium accessible in the rhizosphere under plants of vines, the Codrinski variety. The experience from the vegetable complex

	N, %	P_2O_5, mg/100 g	K_2O, mg/100 g
Control	0.13	4.4	15.0
Az. chroococcum, in soil	0.12	4.0	15.2
Ps. fluorescens, in soil	0.13	4.4	15.2
Az. chroococcum + Ps. fluorescens, in soil	0.12	5.2	15.1
Az. chroococcum + Ps. fluorescens in soil + micoelemente foliar;	0.12	5.2	15.2
Az. chroococcum +Ps. fluorescens, in soil+ microelemente foliar	0.12	6.7	15.1
Az. chroococcum, foliar	0.11	9.6	15.2
Ps. aureofaciens, foliar	0.11	6.7	15.0
Az. *chroococcum + Ps. aureofaciens,* foliar	0.11	1.4	20.2
Az. chroococcum + Ps. aureofaciens + microelements foliar	0.12	8.76	18.1
DL$_{0.5}$	0.1	1.7	0.8

As a rule, the number of phosphorus compounds in the soil is quite high, but most of it is not available for plant nutrition. In addition, a significant part of inorganic phosphorus compounds used as fertilizer is chemically immobilized in a relatively short period and becomes inaccessible to plants, which limits production (Naoko & Wasaki, 2010). Thus, the solubilization and mineralization of phosphorus by phosphate-mobilizing bacteria is an important feature of PGPB. The content of exchangeable potassium increased only in the variants with foliar fertilization with *Az. chroococcum* + *Ps. aureofascens* applied without and with microelements. Analogical effect was mentioned by some researchers (Avis et al., 2008). The soil analysis in the experiment demonstrated insignificant changes in the content of nutritional elements. A statistically significant increase in ammoniacal nitrogen was found in the variant with the application of *Ps. aureofaciens + Az. chroococcum,* foliar + micoelements foliar. A mathematically assured increase in nitric nitrogen concentration has also been demonstrated. Especially for the variants with the application of the suspension of *Ps. fluorescens + Az. chroococcu*m, in soil + microelements, foliar, as well as in the variant with application of metabolites of *Ps. aureofaciens + Az. chroococcum*, foliar. A trend of increasing mobile phosphorus in the plant rhizosphere was observed in the experimental variants. In the specialized literature, it is mentioned that the rhizospheric bacteria, especially the *Azotobacter* and *Pseudomonas* strains, contribute to increasing the amount of nutrients available in the soil. On the other hand, it is known that the increased accumulation of nitric nitrogen (NO_3) in the soil leads to a decrease in the consumption of nitrates by In our variants with the application of rhizobacteria and microelements, the growth of plants was much more intensive compared to the control, correspondingly - and the consumption of nitrates from the soil by the plants, which can explain the decrease in the content of nitric nitrogen in the soil (Martínez-Viveros et al., 2010). The content of exchangeable potassium increased only in the variants with foliar fertilization with *Az. metabolites, chroococcum* + *Ps. aureofaciens* applied without and with micoelements. The data obtained in general coincide with those obtained by some researchers in experiments with other plant species, who established that rhizospheric bacteria in particular contribute to increasing the amount of nutrients in the soil accessible to plants, especially mobile phosphorus.

Table 3. The content of nutrients in the rhizosphere of vine plants, the Presentable variety, the experience from the vegetation complex, mg/100 g soil

Experimental variant	N-NH_4	N-NO_3	P_2O_5	K_2O
Control	0.8	2.58	2.8	24.4
Ps. fluoreiscens + *Az. chrocoocum,* in soil	0.9	3.43	3.0	22.0
Ps. fluorescens + *Az. chroococcum,* in soil + trace elements, foliar	0.9	5.37	3.1	19.2
Ps. aureofaciens + *Az. chrocoocum*, foliar	1.0	6.16	2.9	20.4
Ps. aureofaciens + *Az. chroocoum,* foliar + trace elements, foliar	1.4	3.43	2.9	20.4
$DL_{0.5}$	0.2	1.5	1.2	1.6

The role of microelements in plant metabolism is difficult to overestimate (Gartel, 1974).

They are involved in plant nutrition directly as nutrients and as activators of many metabolic processes. The ionic forms of Fe, Zn, Cu, Mn, B and Ni are part of or act as cofactors in numerous enzymes. The determination of the content of the mobile forms of microelements in the soil of the experimental lot with the developed scheme demonstrated the following tendency: the incorporation of bacterial suspensions into the soil contributed to the decrease of the Fe content, but the foliar application of the metabolites maintained the Fe content at the level of the control (Table 4). Iron is the fourth most abundant element on earth. In aerobic soils, it is poorly absorbed by bacteria or plants, because iron ions, which prevail in nature, are poorly soluble, so their amount, which is available for assimilation by living organisms, is extremely low. And microorganisms and plants need a high level of iron. All bacterial products contributed to the increase of Mn content in the rhizospheric soil; bacterial suspensions and metabolites, applied together with microelements, significantly increased the Zn content in the rhizospheric soil; the content of Cu in the given case is almost in all variants at the level of the control and practically does not depend on the application of fertilizers (Marleny, 2006).

Table 4. The content of microelements in the rhizosphere, the experience from the plant complex, the Codrinski variety, (mg/kg)

Experimental variant	Cu	% control	Fe	% control	Zn	% control	Mn	% control
Control	4.0	100	504.0	100	2.3	100	15.2	100
Az. chroococcum, in soil	4.0	100	448.0	88.9	2.3	100	17.1	112.5
Ps. fluorescens, in soil	4.0	100	392.0	77.8	3.9	169.6	18.7	123.0
Az. chroococcum + *Ps. fluorescens,* in soil	4.6	115	448.0	88.9	2.3	100	22.2	146.1
Az. chroococcum + *Ps. fluorescens* in soil + microelements, foliar	4.0	100	448.0	88.9	7.0	304.3	22.2	146.1
Az. chroococcum + *Ps. fluorescens* + microelements, foliar	4.0	100	448.0	88.9	7.3	317.4	16.4	107.8
Az. Chroococcum, foliar	4.4	112	448.0	88.9	7.0	304.3	19.9	130.9
Ps. aureofaciens, foliar	4.0	100	504.0	100	4.6	200.0	16.4	107.9
Az. chroococcum + *Ps. aureofaciens,* foliar	4.0	100	504.0	100	7.9	343.5	18.7	123.0
Az. chroococcum + *Ps. aureofaciens* microelements foliar	4.0	100	616.0	122.2	2.3	100	17.6	115.8
$DL_{0.5}$	0.1		1.1		2.5		1.5	

It is known that pseudomonads, including *Ps. fluorescens*, in order to survive in conditions with limited reserves of available iron, synthesize soluble yellow-green fluorescent pigments - siderophores, with low molecular weight, molecules with a particularly high affinity for Fe $^{3+}$, as well as membrane receptors capable of binding to siderophore complexes of iron, thus facilitating the absorption of iron by microorganisms (Asada, 1999). Binding of iron to pseudomonad siderophores results in restriction of phytopathogen growth and enhancement of plant growth. According to the data presented, the lowest iron content in the soil was in the variant with the incorporation of the *Ps. fluorescens* (77.8% compared to the control), and the foliar fertilization of plants with a solution of metabolites of *Az. chroococcum* + *Ps. aureofaciens* + microelements, foliar contributed to increasing the iron level in the rhizosphere (by 20% compared to the control). The supply of iron to plants is particularly important under conditions of exposure of plants to stress factors, especially caused by heavy metals. The determination of the content of mobile forms of microelements in the rhizosphere of the cuttings at the end of the experiment highlighted the fact that, when planting the cuttings, the application of the bacterial suspension of *Ps. fluorescence* + *Az. chroococcum* in soil separately and by combining extraradicular fertilization with microelements, foliar caused an increase in the level of Zn in the soil. This fact indicates the increase in the content of accessible forms of these elements for plants (Table 5). Grapevine being a perennial crop, it is very sensitive to the imbalance of microelements, and the rhizospheric bacteria acted as shock absorbers that balanced the trophic stresses in the soil and plants (Fuentes-Ramires & Caballero-Mellado, 2006).

Table 5. The content of accessible forms of microelements in the rhizosphere under the action biofertilizers, mg/kg soil

Experimental variant	Zn	Cu	Fe	Mn
Control	1.6	4.2	175.7	6.4
Ps. fluorescens+ *Az. chroococcum,* in soil	5.9	4.4	186.6	7.4
Ps. fluorescens+ *Az. chroococcum,* in soil + microelements foliar	1.,0	4.2	175.2	6.9
Ps. aureofaciens+ *Az. chrocoocum,* foliar	2.0	5.5	177.7	5.5
Ps. aureofaciens+ *Az. Chroococcum* + microelements, foliar	1.4	4.3	167.8	6.6
$DL_{0.5}$	2.3	0.9	1.4	0.8

The content of microelements in the soil under the plants per fruit in the experiment on the experimental plot was determined in the second half of the vegetation period - over a month after the triple foliar fertilization with bacterial metabolites and microelements. The content of Cu in the soil under the fruit plants was significantly increased, 14.4 and 4.0 mg/kg, due to multiple treatment with preparations containing copper against *Plasmopara viticol*a (Table 6). There is the opinion that it accumulates in the superficial layers of the soil and does not migrate on the soil profile in depth. The data presented show the increased content of Cu on 0-30 cm. Previously, it was demonstrated that the increased share of Cu in the soil is accompanied by the decrease in the content of the accessible forms of microelements Fe and Zn, compared to the soil occupied by annual plants.

Table 6. Content of accessible forms of microelements in soil under grapevines after foliar fertilization, experimental lot, Codrinski variety, mg/kg

Experimental variant	The depth, cm	Cu	% control	Fe	% control	Zn	% control	Mn	% control
Control	0-30	14.40	100	475.0	100	3.46	100	34.55	100
	30-60	8.80	100	409.0	100	3.45	100	34.52	100
Ps. aureofaciens, foliar	0-30	15.20	105.6	382.0	80.4	4.32	124.8	43.20	125.0
	30-60	12.80	145.5	426.0	104.2	4.47	129.6	44.70	129.6
Az. chroococcum, foliar	0-30	12.80	88.9	455.0	95.8	4.47	129.2	44.70	129.4
	30-60	14.00	97.2	455.0	111.2	5.70	165.2	56.95	165.1
$DL_{0.5}$		2.5		3.6		1.2		1.5	

The tendency to decrease the content of Cu in the soil in the variants with Microcom is clearly pronounced, which confirms our assumption about the effect of microelements, prepared with microelements, in reducing the excessive content of this element in the soil under perennial plants.

CONCLUSIONS

Application of suspensions and metabolites of bacteria with plant growth stimulating function, *Az. chroococcum, Ps. aureofaciens* and *Ps. fluorescens* (PGPB) contributes to the more intense accumulation of nutrients by plants, which contributes to the reduction of the erosion process.

The application of the complex of micro-elements and together with the suspensions and metabolites of bacteria with plant growth stimulating function, *Az. chroococcum, Ps. aureofaciens* and *Ps. fluorescens* (PGPB) contributes to increasing the accessibility of nutrients to grapevine plants, due to the bacteria's ability to efficiently dissolve inaccessible forms of nutrients.

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