[1,2]Mykola KHARYTONOV, [1]Oleksandr MYTSYK, [1]Valentina PASHOVA,
[1]Svitlana LEMISHKO, [1]Oleksandr IZHBOLDIN, [2]Laura MEJIAS

SEEDS TREATMENT WITH BIOINOCULANTS FOR WINTER WHEAT CULTIVATION IN THE MARGINAL LANDS

[1]Dnipro State Agrarian and Economic University (DSAEU), Dnipro, UKRAINE
[2]BETA technological center, University of Vic, Vic, SPAIN

Abstract: The highest effect of wheat seeds inoculation in the first field experiment managed in the plain conditions was fixed for Agrobacterium radiobacter strain 204, treatment with Paenibacillus polymyxa impact take second place. In the second field experiment managed in the reclaimed lands, a bigger winter wheat yield can be obtained after the seeds treatment with mycorrhiza (Glomus fasciculatus strain, New Zealand). It is obvious that winter wheat seeds treatment with mycorrhiza leads to partial removal of the phytotoxic effect of underlying rocks on the development of root mass in the arable soil layer.
Keywords: seeds, bio-inoculants, associative nitrogen fixation, soluble phosphorus mobilization, winter wheat, marginal lands

1. INTRODUCTION

The limitations of the crop's yield can be explained by abiotic, biotic, and anthropogenic factors occurrence. As usual marginal lands define as soils that have physical and chemical problems or are uncultivated or adversely affected by climatic conditions. Following this definition erodible, compacted, saline, acid, contaminated, or sandy soils, reclaimed mine soils, urban marginal sites, and abandoned or degraded croplands fit this term (*Blanco-Canqui 2016*). However, it is necessary to raise agricultural productivity without enhancing the environmental footprint (*Di Benedetto et al., 2017*).Seed inoculation has been considered a precise and cost-effective method to deliver microbial inoculants (*Ehsanfar and Modarres-Sanavy, 2005; O'Callaghan, 2016*), with the potential for large-scale bio-fertilizers application in the conditions of crossing relief to improve crops associative nitrogen fixation in the root zone, and soil phosphorus mobilization. *Rhizobium radiobacter* (syn. *Agrobacterium tumefaciens,* syn. "*Agrobacterium fabrum*") is an endofungal bacterium that induces growth promotion and systemic resistance in cereal crops, including wheat (*Guo et al, 2017*). Recently, biological seed coating with PGPM was proposed as an alternative to conventional seed treatment (such as fertilizer and protection products) to mitigate biotic and abiotic stresses. (*Ma, 2019*). The seed coating process involves covering seeds with a small number of exogenous materials to deliver beneficial microbes to crops (*Rocha et al., 2019a*). The improved growth, yield, and nutrient uptake in wheat plants demonstrated the potential of mycorrhizal inoculation to reduce the effects of drought stress on wheat grown under field conditions in semiarid areas (*Al-Karaki et al., 2004*). The arbuscular mycorrhiza symbiosis with crop roots increased with rising soil temperatures in the spring, in time to enhance late-sea-son P accumulation and grain production (*Mohammad et al, 1998*). Field AMF inoculation increased aboveground biomass, grain yield, harvest index, aboveground biomass P concentration, and content, straw P content, aboveground biomass N concentration and content, grain N content, and grain Zn concentration (*Pellegrino et al., 2015*).

The main objective of our work was to study the effects of seed inoculation with plant growth stimulants applied for crop cultivation in marginal lands.

2. MATERIALS AND METHODS

Unsteady agriculture in the steppe zone of Ukraine is in most cases by rather changeable weather conditions relief and diversity of soil covering. Precipitation is less than 350mm in dry years i.e. in dry steppe zone, but in damp years more 550mm live in forest-steppe zone (average perennial value 470 mm).The relief makes worse the influence of the zonal climate, with redistribution climatic ecological recourses leads to draining of soil formation of soil erosion, deterioration of fertility of it. Thus, erosion processes within steppe landscapes reached 40-50%. The negative balance of the soil nutrition elements reaches 100 kg per hectare and more. It is necessary to emphasize that black soils are prevalent in the steppe zone of Ukraine (*Kharytonov et al., 2004*). Generally, black soils are not rich in available phosphorus. They have available K content ranging from medium to high. The reduction of fertilizer inputs leads to nutrition balance infringement and the development of degradation processes in the black soil. Field observations were

ISSN 1584 – 2665 (printed version); ISSN 2601 – 2332 (online); ISSN-L 1584 – 2665

carried out at the ecological field station of the scientific- advance farm located in the Slobozhansky district of Dnipropetrovsk province in the northern part of the steppe zone of Ukraine (Figure1).

The farm coordinates are 48°30'N lat. and 35°15' E long. The research field was used for many years as an area for intensive agricultural production and research (*Kharytonov et.al, 2016a; Kharytonov et. al, 2016b*). The research field occupies an area of 14 hectares and it is crossed by three ravines. One of them is of 30 m depth with a slope of > 7°, the other two have slopes up to 3°.

Figure 1 - Illustration of the scheme of research on the scale of district-farm-field

Special attention was paid to one of the three ravines: flat terrain, slopes of the southern and the northern exposure. Thus, the experiments reported here compare soils from three types of landscapes: level soils (0-1% slope) with no observable erosion (E0), mildly sloped soils (1-3%) with mild erosion (E1, 0-10 cm topsoil loss), and moderately sloped soils (5-7%) with moderate erosion (E2, up to 30 cm topsoil loss). Coincidentally, in the study area, the mildly sloped soils have the northern exposure, and the moderately sloped soils have the southern exposure. The soil type in the experimental area is referred to as the central steppe chernozem (black soil) in the FSU (former the Soviet Union) system (*Kravchenko et al., 2010*).

The soil nitrification energy was determined by the following procedure. A portion of a bulk sample for each landscape point was allocated to each of two 50 ml Erlenmeyer flasks. NO_3 concentration was determined for each flask immediately after the soil sampling, and again after 7 days of incubation at 28 C^o with daily addition of water just enough to keep the soil moisture for the period of the experiment. NO_3 concentration was determined using an Ion-meter and a nitrate selective ion electrode. Using 4 equally-spaced concentrations of KNO_3 from 10^{-2} to 10^{-5} M (approximately equivalent to 0.78 to 780 mg NO_3/kg of solution), a standard curve was prepared. For greater accuracy, this curve was divided into two segments (10^{-2} to 10^{-4} M and 10^{-4} to 10^{-5} M). Mobile P was determined by the colorimetric method of Denizhe, following offered by the Michigan extraction method. The method of Michigan is basically an extraction by a 1% solution of ammonia (*Kharytonov et al, 2016b*). The microbiological activity of the soil samples taken according to landscape differentiation in the territory of the ecological field station of DSAEU was studied.

The second case study was conducted on the basis of the Pavlograd land reclamation station for reclamation situated in Western Donbas (eastern Ukraine). The station coordinates 48°33'24'' N, 35°58'46'' E). The station was founded in the floodplain of the Samara River in order to examine the best restoration measures (*Klimkina et al, 2018*). In the following map part of the emplacement of the agricultural lands occupied by land reclamation station is shown (Figure 2).

Figure 2 - Aerial map of the coal mine emplacement at Donbas district (48°33'32"N 35°59'13"E).

The main reclamation objective included the cultivation of field grain and energy crops. The scheme for reclamation of disturbed land was based on the study of the effectiveness of capping the mine dumps with different layers of black-soil mass both with and without a shielding layer of loess-like loam. The following artificial models (variants) of technogenic edaphotops were used to look into the peculiarity of upward migration of heavy metals from the coal mine dump: mine rock (MR) + 30 cm of the bulk layer of black soil (30BS); MR + 50BS; MR + 50BS; MR + 50 cm of the loess-like loam (50LLL) + 30BS; MR + 50LLL + 50BS; MR + 50LLL + 70BS. The GIS maps were created in the ArcMap 9.3.1 application of ESRI's ArcGIS Desktop GIS

ISSN 1584 – 2665 (printed version); ISSN 2601 – 2332 (online); ISSN-L 1584 – 2665

software, using SAS Planet version 141212.8406. Interpolation methods were used to build GIS maps in the ArcMap software component and to assume intermediate values of raster points based on the available discrete set of known values.

Bacterial fertilizers *Agrobacterium radiobacter 20, Bacillus polymyxa KB* and two strains of *Glomus fasciculatus* were cultivated at the Institute of Agroecology and Nature Management UAAS. Winter wheat variety Albatros Odesky was taken as the object of field experiments.

3. RESULTS

GIS maps built according to GPS data and soil observation of the territory of the research field of the DSAEU regarding the level of nitrification energy and mobile phosphorus are shown in Figure 3.

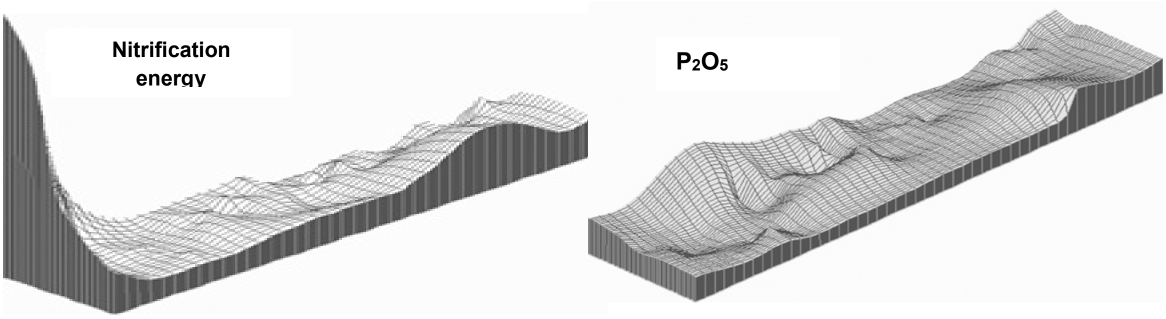

Figure 3 - GIS maps of soil nitrification energy and mobile phosphorus distribution in the crossing relief

The high heterogeneity of the nutrition elements (nitrogen and phosphorus) distribution over the studied area was established. In nitrification processes the microbial functional importance changes depending on the soil nutrition status in each place (*Di Benedetto et al., 2017*). The presence of variability of chemical elements in the conditions of rugged terrain makes it necessary to distinguish types of land for the further development of systems of differentiated application of appropriate forms of fertilizers. Meanwhile, the preliminary seeds treatment with bio-inoculants helps to increase the adaptive potential of crops to the deficiency of nutrients (*Kharytonov and Resio Espejo, 2013).* It was reported earlier that winter wheat cultivars demand different relief conditions and problems of nutrition element losses (*Kharytonov et al, 2017*). Studies were performed on plain (full-height normal soil), on the northern exposition slope (low eroded soil), and on the slope of the southern exposure (middle level of erosion). Special attention was paid to one of the three ravines: flat terrain, slopes of the southern and the northern exposure. It was established that the north exposition of the slope made up more favorable conditions for wheat vegetation. To make this information clear we measured the number of ammonifiers and oligonitrofiles (Figure 4).

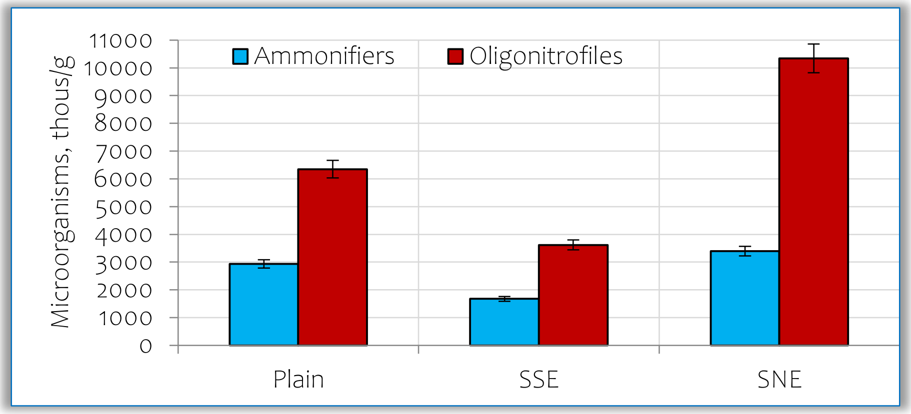

Figure 4 - The content of ammonifiers and oligonitrofiles in the top soil depending on relief, thous/g

The ammonifiers and oligonitrofiles quantity distributed in the line: the slope of northern exposition, plain, and slope of southern exposition. These data confirm that more favorable conditions for crop cultivation in the crossing relief are connected with larger level of soil moister on the slope of the northern exposition. That is why seed inoculation and coating have been proposed as promising tool for inoculation of different crop seeds since it is able to use minor amounts of inocula in a precise application (*Accinelli et al., 2018; Rocha et al., 2019b*). The main types of seed coatings include seed dressing, film coating, and pelleting, which can be chosen differently, according to the purpose of application and the type of seed or selected microbes.

ISSN 1584 – 2665 (printed version); ISSN 2601 – 2332 (online); ISSN-L 1584 – 2665

The purpose of the following field experiment managed on a flat area was to establish the effectiveness of winter wheat seed treatment with *Agrobacterium radiobacter strain* 204" and *Paenibacillus Polymyxa* inoculants (Figure5).

The highest effect of seeds inoculation was fixed for *Agrobacterium radiobacter* strain 204, treatment with Paenibacillus polymyxa impact take second place. The pre-sowing bacterization with agrobacterium radiobacter 204 contributed to the increase of the potential nitrogen-fixing activity of soil rhizosphere in other field experiments managed in the forest-steppe zone of Ukraine (*Kozar et al., 2017*). The taxonomic position of "*Agrobacterium radiobacter* strain 204," used as a cereal crop growth promoting inoculant, was derived by a polyphasic approach (*Humphry et al, 2007*). It is concluded that strain 204 is phenotypically and genotypically very similar to the current *R. radiobacter* type strain and that the mechanism of its effect on the growth of cereals is via the production of plant growth-promoting substances.

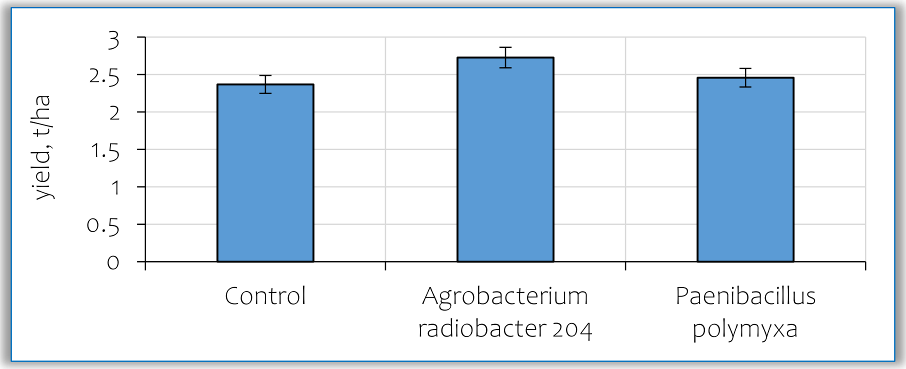

Figure 5 - The seed treatment impact with bacterial fertilizers on the winter wheat yield, t/ha.

GIS maps of layer-by-layer assessment of pH distribution over the area of six experimental plots in the Pavlograd land reclamation station are shown in Figure 6.

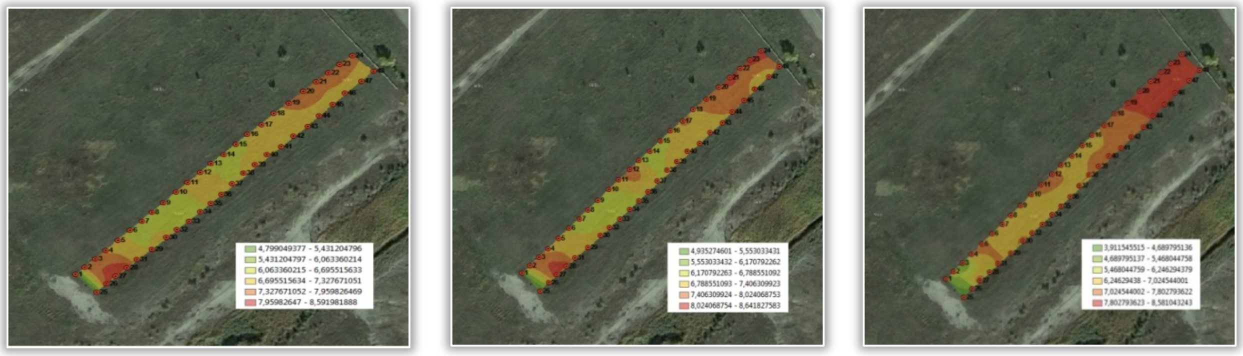

Figure 6 - Layer-by-layer distribution of pH in the black soil over the area of six reclamation profiles
(a - 0-10cm; b - 10-20cm; c - 20-30cm)

The 2D soil pH change is connected with the movement of water-soluble salts from the dumped mine rocks to the upper horizons of the artificial reclamation.

The field experiments at the Pavlograd land reclamation station of DSAEU established the effect of a higher level of winter wheat yield in the trials with a protective layer of loess-like loam (7 and 8).

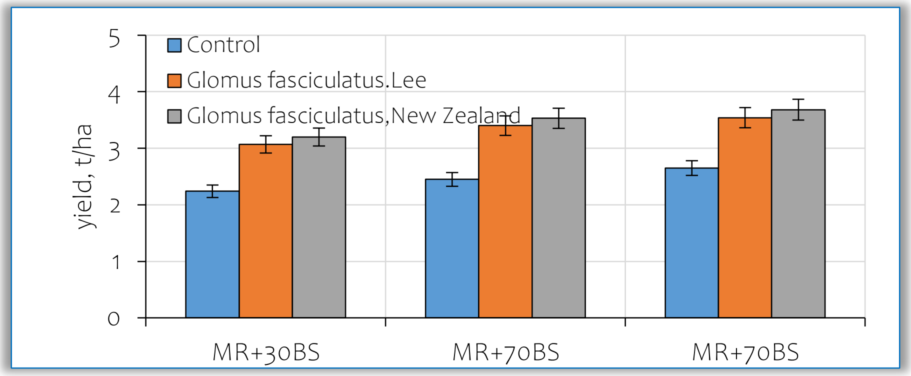

Figure 7 - The mycorrhiza seeds treatment effect on the yield of winter wheat in reclamation option without a layer of loess loam, t/ha

ISSN 1584 – 2665 (printed version); ISSN 2601 – 2332 (online); ISSN-L 1584 – 2665

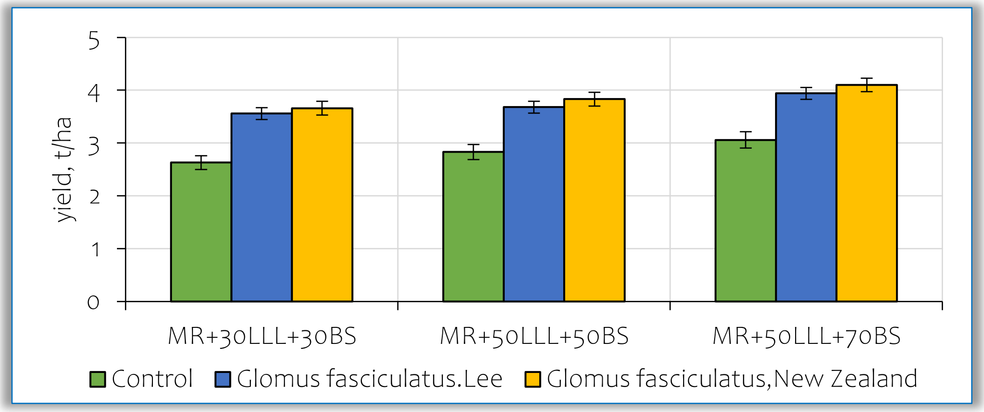

Figure 8 - The mycorrhiza seeds treatment effect on the yield of winter wheat in reclamation option with a layer of loess loam, t/ha

The obtained results show that a bigger winter wheat yield can be obtained after the seeds treatment with mycorrhiza *Glomus fasciculatus, New Zealand*. It is obvious that winter wheat seeds treatment with mycorrhiza leads to partial removal of the phytotoxic effect of underlying rocks on the development of the root mass in the arable soil layer.

4. CONCLUSIONS

Two field experiments with winter wheat seeds treatments by bio-inoculants were managed in erodible and reclaimed mine lands. Special attention in the first case study was paid to one of the three ravines: flat terrain (plain), slopes of the southern and the northern exposure. It was established that the north exposition of the slope made up more favorable conditions for wheat vegetation. The amonifiers and oligonitrofiles quantity in topsoil distributed in the line: the slope of northern exposition, plain, and slope of southern exposition. These data confirm that more favorable conditions for crop cultivation in the crossing relief are connected with a larger level of soil moister on the slope of the northern exposition. The highest effect of wheat seeds inoculation in the field experiment managed in the plain conditions was fixed for *Agrobacterium radiobacter* strain 204, treatment with *Paenibacillus polymyxa* impact take second place. In the second field experiment managed in the reclaimed lands, a bigger winter wheat yield can be obtained after the seeds treatment with mycorrhiza *Glomus fasciculatus, New Zealand*. It is obvious that winter wheat seeds treatment with mycorrhiza leads to partial removal of the phytotoxic effect of underlying rocks on the development of root mass in the arable soil layer.

Acknowledgement

This study was supported by the Ministry of Education and Sciences of Ukraine.

Note: This paper was presented at ISB–INMA TEH' 2022 – International Symposium on Technologies and Technical Systems in Agriculture, Food Industry and Environment, organized by University "POLITEHNICA" of Bucuresti, Faculty of Biotechnical Systems Engineering, National Institute for Research–Development of Machines and Installations designed for Agriculture and Food Industry (INMA Bucuresti), National Research & Development Institute for Food Bioresources (IBA Bucuresti), University of Agronomic Sciences and Veterinary Medicine of Bucuresti (UASVMB), Research–Development Institute for Plant Protection – (ICDPP Bucuresti), Research and Development Institute for Processing and Marketing of the Horticultural Products (HORTING), Hydraulics and Pneumatics Research Institute (INOE 2000 IHP) and Romanian Agricultural Mechanical Engineers Society (SIMAR), in Bucuresti, ROMANIA, in 6–7 October, 2022.

References

[1] Accinelli, C., Abbas, H. K., Little, N. S., Kotowicz, J. K., Mencarelli, M., & Shier, W. T. (2016). A liquid bioplastic formulation for film coating of agronomic seeds. Crop Prot. 89, 123–128
[2] Al-Karaki, G., McMichael, B., & Zak, J. (2004) Field response of wheat to arbuscular mycorrhizal fungi and drought stress. Mycorrhiza 14, 263–269
[3] Blanco-Canqui, H. (2016). Growing Dedicated Energy Crops on Marginal Lands and Ecosystem Services. Soil Sci. Soc. Am. J. 80, 845–858
[4] Di Benedetto, N.A., Corbo, M.R., Campaniello, D., Cataldi, M.P., Bevilacqua, A., Sinigaglia M., & Flagella Z. (2017). The role of Plant Growth Promoting Bacteria in improving nitrogen use efficiency for sustainable crop production: a focus on wheat. AIMS Microbiol. 3(3), 413-434
[5] Ehsanfar, S., & Modarres-Sanavy, S. A. (2005). Crop protection by seed coating. Commun. Agric. Appl. Biol. Sci. 70, 225–229.
[6] Guo H., Glaeser S.P., Alabid I., Imani J., Haghighi H., Kämpfer P., & Kogel K.-H. (2017) The Abundance of Endofungal Bacterium Rhizobium radiobacter (syn. Agrobacterium tumefaciens) Increases in Its Fungal Host Piriformospora indica during the Tripartite Sebacinalean Symbiosis with Higher Plants. Front. Microbiol. 8:629
[7] Humphry, D.R., Andrews, M., Santos, S.R. et al. (2007). Phylogenetic assignment and mechanism of action of a crop growth promoting Rhizobium radiobacter strain used as a biofertiliser on graminaceous crops in Russia. Antonie van Leeuwenhoek 91, 105–113
[8] Kharytonov, M., Bagorka, M., & Gibson, P. (2004). Erosion effects in the central steppe chernozem soils of Ukraine// I.Soil Properties. Agricultura. Published by the University of Maribor, Faculty of Agriculture, Slovenia. - Vol.3. - № 1, 12-18.

[9] Kharytonov, M. M., & Resio Espejo, J.M. (2013) Prospects of the Nikopol manganese basin rocks using for land reclamation. Ecology and noospherology. 2013. Vol. 24, no. 1–2, 81-90

[10] Kharytonov, M.M., Pashova, V.T., Bagorka, M.O., Kozechko, V.I., & Dudar, T.O. (2016a): Arable lands degradation in the northern steppe zone of Ukraine. Agriculture and Forestry, 62 (2), 71-80.

[11] Kharytonov, M.M., Pashova, V.T., Bagorka, M.O., & Kozechko, V.I. (2016b). Nutrition regimes of eroded lands in the northern steppe zone of Ukraine. Agriculture and Forestry, 62 (3), 175-185

[12] Kharytonov, M.M., Pashova, V.T., Mitsik, O.O., Nazarenko, M.M., & Bagorka, M.O. (2017). Estimation of winter wheat varieties suitability for difference growth of landscape conditions. ANNALS of Faculty Engineering Hunedoara – International Journal of Engineering, 187-191.

[13] Klimkina, I., Kharytonov, M., & Zhukov, O. (2018).Trend Analysis of Water-Soluble Salts Leaching Along Surfaces of Reclaimed Mine Dumps in Western Donbass (Ukraine). Environmental Research, Engineering and Management, Vol. 74, No 2, 82-93

[14] Kozar, S. F., Evtushenko, T. A., Usmanova, T. O., & Symonenko, Ye. P. (2017). Microbiocenosis of rhizospheric soil and productivity of winter wheat under the use of agrobacterium radiobacter 204, activated with nanocarboxylates of metals. Agricultural Microbiology. 26:17-23

[15] Kravchenko, Y., Perenko, L., & Zhang, X.Y. (2010) Ukrainian Chernozems: genesis, properties and amendment. in Book: New advances in research and management of world Mollisols/X. B. Liu, C. Y. Song, R. M. Cruse, and T.Huffman, eds. Northeast Forestry University Press, p.3 – 24. Harbin, China

[16] Mohammad, M.J., Pan, W.L., & Kennedy, A.C. (1998). Seasonal mycorrhizal colonization of winter wheat and its effect on wheat growth under dryland field conditions. Mycorrhiza, 8, 139-144.

[17] O'Callaghan, M. (2016). Microbial inoculation of seed for improved crop performance: issues and opportunities. Appl. Microbiol. Biotechnol. 100, 5729– 5746

[18] Pellegrino, E., Öpik, M., Bonari, E., & Ercoli, L. (2015). Responses of wheat to arbuscular mycorrhizal fungi: A meta-analysis of field studies from 1975 to 2013. Soil Biology and Biochemistry, Vol.84, pp.210-217

[19] Rocha, I., Ma, Y., Souza-Alonso, P., Vosátka, M., Freitas, H., & Oliveira, R.S. (2019a) Seed Coating: A Tool for Delivering Beneficial Microbes to Agricultural Crops. Front. Plant Sci. 10:1357

[20] Rocha, I., Ma, Y., Carvalho, M. F., Magalhães, C., Janoušková, M., Vosátka, M., et al. (2019b). Seed coating with inocula of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria for nutritional enhancement of maize under different fertilization regimes. Arch. Agron. Soil Sci. 65, 31–43

ISSN 1584 – 2665 (printed version); ISSN 2601 – 2332 (online); ISSN–L 1584 – 2665