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RESEARCH ARTICLE

STUDY ON IMPACT OF MINERAL FERTILIZERS ON BIOGENITY AND ENZYMATIC ACTIVITY OF SOILS WITH COMMON WHEAT.

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Abstract

The improving of the soil fertility requires the determining of appropriate fertilizer variants, easily assimilated and quickly acting, for obtaining of qualitative and high-yield agricultural produce. The purpose of the present development is to be researched the impact of mineral fertilizer products of „Agropolychim“ AD on the soil microbiocenosis in the rhizosphere of wheat and on the catalytic and cellulase activity of the fertilized soils. It is determined, that the biogenity of the soils is increased at fertilizing in comparison with the control (unfertilized) samples. The used fertilizers increase the cellulase activity with all variants, while the catalytic activity is higher with the combined fertilizing with urea and ammonium nitrate, as well as with the control samples. The studied microbiological and enzyme indicators can serve as sensitive biological and biochemical indicators with fertilized soils.

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Introduction:-

The fertilizing of the soils is of essential significance for improvement of their fruitfulness, which leads to increase of the yields and quality of the agricultural produce, and hence to the animals' and persons' feeding of full value. The usage of fertilizers must be conformed to the norms for fertilizing, thus to be preserved stable ecological equilibrium in nature, the soils not to be polluted, the underground waters and vegetation, because this would have a negative effect over human's health.

The applying of organic and mineral fertilizers increases the abundance of microorganisms and to a certain degree the enzyme activity of the soils (Li et al., 2008). Other studies show, that the usage of big doses mineral fertilizers changes the composition and the quantity of the soil microorganisms (Monokrousos et al., 2006). Iovieno et al. (2009) has determined, that the fertilizing with organic and mineral fertilizers improves the microbiological activity of the soil and creates cumulative effects, increases the activity of the enzymes phosphatase and aryl-phosphatase, which supposes the usefulness of the repeated applications of the fertilizers. The bringing in of mineral fertilizers has a stimulating effect for the ammonifying bacteria, while the actinomycetes and bacteria, absorbing mineral nitrogen are decreasing (Bogdanov et al., 2015).

The bringing in of nutritive substances in the soils through the fertilizing affects not only the plants, but also the connected with them microbial communities in the soil. According to Geisseler and Scow (2014) the application of

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mineral fertilizers leads up to 15.1% increase of the microbe biomass in comparison with unfertilized soils. The mineral fertilizing increases the content of organic carbon in the soil and it is a main factor, contributing to the total increase of the quantity of microorganisms with mineral fertilizing. The effect of the fertilizing over the soil microflora is also dependent on pH – the quantity of the microorganisms decreases with fertilized soils with pH under 5 and increases at higher pH values of the soil. The applying of urea and ammonium fertilizers, however, can temporarily increase pH, the osmotic potential and the concentrations of ammonia up to levels, inhibiting the microbial communities. The duration of the fertilizing also influences the microbial biomass, as its increase is most expressed at studies with duration at least 20 years (Geisseler и Scow, 2014). At fertilizing with mineral fertilizers and combined fertilizing with mineral and organic fertilizers of soils under wheat and maize for a 15-year long-term experiment Li et al. (2008) have determined, that the fertilizing increases the microbial biomass and the ureasic activity of the soils, as they have determined positive correlation between these two indexes and the nutritive substances in the soil. While the subordination between the activity of the catalase and the nutritive substances in the soil is not substantial. Other authors have determined, that the activity of the catalase is increased at fertilizing with up to 7,5 ml fertilizer NPK (concentration 250 mg/ml), and with increasing of the dose up to 12,5 ml decreases the enzyme's activity (Okore et al., 2014). The same doses and concentrations have not suppressed the development of bacteria and micromycetes according to studies of these authors, and the dehydrogenase activity, the acid and alkaline phosphatase are suppressed at the beginning of the experiment, but subsequently the activity of these enzymes is increased. The prolonged usage of fertilizer systems (mineral, organic, combined usage of mineral and organic fertilizers) in comparison with unfertilized soil, assists the increasing of the total number of microorganisms, nitrogen-fixing and nitrifying bacteria, as well as for increasing the activity of the enzyme cellulase in soils (Kosolapova et al., 2016). According to a number of studies the combined fertilizing with natural fertilizer and mineral fertilizers has positive as well as negative effects over the soil enzymes (Dick, 1988; Dick, 1992; Kandeler et al., 1999; Olander and Vitousek, 2000).

Among different studied indexes the microbiological and enzyme activity are suggested by number of authors as universal indexes of the soil fertility and pollution (Dilly et al., 2003; Nannipieri et al., 2000; Li et al., 2008; Perucci, 1992; Pascual et al., 1999; García-Gil et al., 2000; Ros et al., 2003; Crecchio et al., 2004; Bastida et al., 2008; Marcote et al., 2001; Malcheva, 2014a,b). The biochemical indexes are most sensitive towards the changes in the soil properties and their measuring are used largely as indicators for the management effect over the condition of the soil and agricultural productivity (Bandick and Dick, 1999; Nayak et al., 2007).

Therefore the topic for a study on the impact of mineral fertilizers on the soil microbiocenosis and enzyme activity of fertilized soils is very interesting and useful. An object of the present report is analyzing the impact of different fertilizer products on the biogenity and enzyme activity of soils with wheat.

Material and Methods:-

The experiment is brought out on a training-experimental field of “Plant production” department to Technical University – Varna. The experiment is set in two repetitions, as there has been used winter common wheat variety „Andino“ (selection of company „Limagrain“). The sowing of the wheat was carried out on 06 October 2016 with norm 600 seeds/m² and size of the experimental plot 4 m².

There have been set two repetitions of the following variants:

Control (unfertilized)

Ammonium nitrate (NH₄NO₃) (34,5% nitrogen)

- Urea (46% nitrogen) + Ammonium nitrate (34,5% nitrogen)

- UAN (Liquid nitrogen fertilizer) (32% nitrogen)

With variants 2, 3 and 4 have been used MAP (pre-sowing) and P:K 16:16 (autumn during phase tillering). Before setting the experiment, during the vegetation and after the harvesting of the crop have been taken soil samples for each of the variants for carrying out of:

1. microbiological analysis of the soil samples, which includes determining of non-spore-forming bacteria, actinomycetes, micromycetes, bacillary microflora, bacteria, assimilating mineral nitrogen;
2. determining the enzyme activity of the soil through studying the enzymes catalase and cellulose.

The microbiological and enzyme analyses have been carried out in a microbiological laboratory at the University of Forestry – Sofia. The samples have been analyzed up to 48 hours after sampling in sterile paper bags, as during this time they have been stored in a refrigerator at temperature 4-10 °C. There has been used the method of the utmost

thinnings and subsequent cultures on solid culture media (mesopeptonic agar for bacilli and non-spore-forming bacteria, starch-ammonium agar for actinomycetes and bacteria, assimilating mineral nitrogen, Czapek Dox agar for mildew fungi). The number of the colony-forming units for each studied group of microorganisms is determined, as the results are recalculated for 1 gr. absolutely dry soil.

The catalytic activity of the soils is determined as per a manganese-metrical method (Khaziev, 1976). The cellulase activity of the samples is studied through a laboratory experiment (Khaziev, 1976). In Petri dishes is sprinkled soil with thickness around 7 mm, as there is maintained 60 % utmost field capacity of moisture. On the soil are put 3 bands sterile filter paper with sizes 10/50 mm. Cultivation in a thermostat at 25 °C. In 15 days is reported percentage decomposed area with the assistance of a net-standard. The statistical analysis of the data includes determining of an average value (out of three repetitions), a coefficient of variation and standard deviation.

Results and Discussion:-

The soil microflora of the studied samples is represented through determining in seasonal dynamics of non-spore-forming bacteria, bacilli, micromycetes, actinomycetes and bacteria, assimilating mineral nitrogen (**Table 1**).

Table 1:-Qualitative composition and quality of the microorganisms (KOE x 103 / gr. abs. dry soil) ± CV; (%)

№	Non-spore forming bacteria	Bacilli	Micro-mycetes	Actino-mycetes	Bacteria, assimilating mineral nitrogen	Total micro-flora	Coefficient of mineralization
<i>Control before setting the experiment</i>							
Control (unfertilized)	5472.0 ± 1.316 (79.4)	936.0 ± 1.068 (13.6)	342.0 ± 2.924 (5.0)	144.0 ± 5.556 (2.1)	3960.0 ± 1.515	6894.0	0.62
<i>Tillering stage</i>							
Control	2889.6 ± 0.163 (71.8)	567.6 ± 0.493 (14.1)	86.0 ± 0.465 (2.1)	481.6 ± 0.083 (12.0)	6914.4 ± 0.064	4024.8	2.00
Urea+NH ₄ NO ₃	4760.0 ± 0.210 (73.7)	901.0 ± 0.111 (13.9)	187.0 ± 0.535 (2.9)	612.0 ± 0.163 (9.5)	1683.0 ± 0.119	6460.0	0.30
NH ₄ NO ₃	2720.0 ± 0.368 (65.3)	986.0 ± 0.351 (23.7)	102.0 ± 0.784 (2.4)	357.0 ± 0.202 (8.6)	2703.0 ± 0.078	4165.0	0.73
UAN	2620.8 ± 0.164 (73.2)	487.2 ± 0.869 (13.6)	168.0 ± 0.315 (4.7)	302.4 ± 0.499 (8.5)	1444.8 ± 0.152	3578.4	0.46
<i>Maturity stage</i>							
Control	2563.2 ± 0.014 (34.6)	4289.8±0.012 (57.9)	71.2 ± 0.743 (1.0)	480.6 ± 0.438 (6.5)	3702.4 ± 0.011	7404.8	0.54
Urea+NH ₄ NO ₃	4888.8 ± 0.041 (35.0)	8225.6±0.039 (58.9)	174.6 ± 0.458 (1.3)	659.6 ± 0.189 (4.7)	5432.0±0.018	13948	0.46
NH ₄ NO ₃	2638.4 ± 0.069 (27.6)	6440.8±0.030 (67.3)	97.0 ± 0.825 (1.0)	388.0 ± 0.258 (4.1)	3414.4 ± 0.062	9564.2	0.38
UAN	2195.2 ± 0.096 (25.4)	6017.2±0.030 (69.6)	98.0 ± 0.408 (1.1)	333.2 ± 0.240 (3.9)	3606.4 ± 0.044	8643.6	0.44
<i>After harvesting stage</i>							

Control	3259.2 ± 0.037 (71.8)	640.2 ± 0.317 (14.1)	97.0 ± 0.412 (2.1)	543.2 ± 0.266 (12.0)	9622.4 ± 0.025	4539.6	2.47
Urea+NH ₄ NO ₃	4326.2 ± 0.018 (64.1)	1552.0±0.515 (23.0)	155.2 ± 0.644 (2.3)	717.8 ± 0.418 (10.6)	1610.2 ± 0.112	6731.8	0.27
NH ₄ NO ₃	5490.2 ± 0.031 (81.6)	698.4 ± 0.359 (10.4)	155.2 ± 0.515 (2.3)	388.0 ± 0.258 (5.8)	9855.2 ± 0.019	6751.2	1.59
UAN	4233.6 ± 0.025 (74.5)	901.6 ± 0.089 (15.9)	176.4±0.300 (3.1)	368.6 ± 0.249 (6.5)	6899.2 ± 0.041	5660.8	1.34

The biogenity of the soils is increased at fertilizing in comparison with the control (unfertilized) samples – up to 2 times, as the increase is highest at the combined fertilizing with urea and ammonium nitrate in the phase of ripening. The addition of nutritive substances in the soil obviously increases the quantity of the microorganisms, but not and their mineralization activity – the speed of decomposing of the organics is lower after fertilizing than before fertilizing. Consequently the bringing in of chemical, nutritive elements in the soil on the one hand leads to increase of the number of the microorganisms and changes in their qualitative composition, but on the other hand it suppressed their activity in a certain moment of oversaturation with different chemical elements and organic substances and „they fall into condition of stress“.

It is determined, that the total microflora is highest with the combined fertilizing with urea and ammonium nitrate (two times in comparison with the control), followed by the fertilizing only with ammonium nitrate, and the total quantity of the microorganisms is lowest with the usage of the fertilizing preparation UAN.

At phase of complete ripeness it makes impression, that out of the ammonifying group of microorganisms (non-spore-forming bacteria and bacilli) higher percentage share occupy the bacilli, which are spore-forming and can endure better unfavourable conditions. The largest difference between the percentages of the bacilli and the non-spore-forming bacteria in favour of the first group – 44,2 % are determined namely with the fertilizing with UAN, where the total quantity of the microorganisms is lowest – probably their development is suppressed. Not always lower quantity of microorganisms means, that their activity is weaker. Besides the reserve with nutritive substances an impact is exercised by the moisture, temperature, pH, mechanical composition of the soil and other factors. A similar tendency is not observed during phase of tillering and harvesting – during these phases the quantity of non-spore-forming bacteria is higher than the one of the bacilli. The described tendency determines and the seasonal dynamics regarding the total microflora – its quantity is higher during the summer in comparison with spring and autumn.

The quantity of bacteria, assimilating mineral nitrogen is highest with the control in first and third phase, and with the combined fertilizing with urea and ammonium nitrate at the phase of complete ripeness, and lowest with the fertilizing only with ammonium nitrate at the same phase and at the usage of UAN for the other two phases. The higher quantity of bacteria, assimilating mineral nitrogen and lower of ammonifying bacteria determines the higher values of the mineralization coefficient (higher speed of decomposition of the organic substances).

Regarding the seasonal dynamics impact render the changing moisture, temperature of the soil and the decreasing reserve with nutritive elements after harvesting. The values of pH do not render impact for the changes of the quantity of microorganisms in the separate seasons, since they show neutral reaction at all studied phases.

The highest biogenity is determined in phase of complete ripeness (but not the highest mineralization activity), while at phase of tillering and harvesting the values of the total microflora are lower and similar, but the speed of mineralization is higher (except for at the fertilizing with urea). The higher total quantity at the phase of ripening is because of the higher quantity of bacilli. Obviously this „transitional state“ favours the development of the microorganisms, especially of the spore-forming, which would survive even more unfavourable conditions. As a whole there is determined higher quantity during the summer because of the stronger development of species surviving in unfavourable conditions under the form of spores (the bacilli).

The results of the catalytic activity of the soil microorganisms with the studied samples are presented in **Table 2**.

A higher catalytic activity with the controls and combined fertilizing with urea and ammonium nitrate is determined, and the activity of the enzyme is lowest with the fertilizing with UAN.

These results correlate with the obtained results about the biogenity of the studied samples – at higher quantity of microorganisms is reported a higher catalytic activity, i.e. besides the physicochemical conditions of the environment significance for increasing the activity of the enzyme renders also the quantity of the microorganisms – a fact, which has not been confirmed about the mineralization activity.

The values of the catalytic activity are around 1,5 times higher in all studied phases, with the controls, as well as with the fertilized soils, in comparison with the control before beginning of the experiment. This fact shows that the catalytic activity depends not only on the fertilizing, but also on many other factors, influencing separately and in complex – the quantity of the microorganisms, appropriate moisture, temperature, reserve with nutritive substances of the soils, as well as on the type of vegetation.

Regarding the catalytic activity studied in seasonal dynamics, the values of the enzyme are higher during the spring in comparison with summer and autumn.

Table 2:-Catalytic activity of the soil microorganisms

№	Crop	Variants	ml O ₂ /30 min. Mean ± SD
1	Control (unfertilized)	Without fertilizer, before beginning of the experiment	2,90 ± 0,09
1	Wheat (tillering stage)	Control-unfertilized	5,10 ± 0,10
2		Urea+NH ₄ NO ₃	5,00 ± 0,05
3		NH ₄ NO ₃	4,85 ± 0,05
4		UAN	4,57 ± 0,03
1	Wheat (maturity stage)	Control-unfertilized	4,87 ± 0,06
2		Urea+NH ₄ NO ₃	4,93 ± 0,06
3		NH ₄ NO ₃	4,52 ± 0,03
4		UAN	4,22 ± 0,03
1	Wheat (after harvesting stage)	Control-unfertilized	5,12 ± 0,08
2		Urea+NH ₄ NO ₃	4,92 ± 0,03
3		NH ₄ NO ₃	4,70 ± 0,09
4		UAN	4,18 ± 0,06

The cellulase activity is higher with the fertilized soils in all phases in comparison with the controls (**Figure 1**).

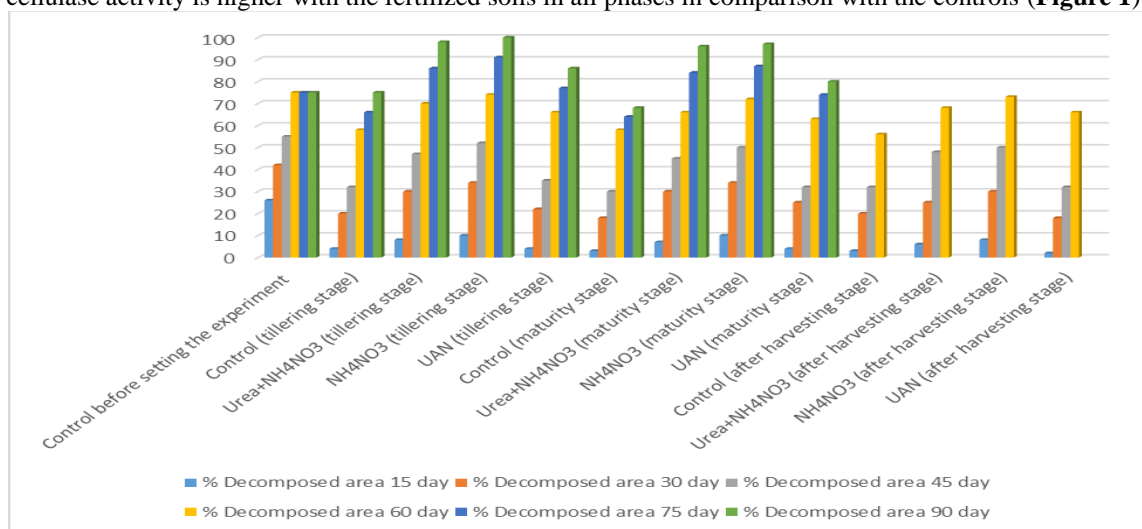


Fig. 1:-Cellulase activity of the soil microorganisms.

The fertilizing increases the cellulase activity with up to 25% during phase tillering (reporting in 3-th month of setting), with up to 29% at phase ripening (reporting in 3-th month of setting) and with up to 17% after harvesting (reporting in 2-nd month of setting) in comparison with the control samples, which is determined and by the fact that during the first two phases the moisture and the reserve with nutritive elements at the studied soils are higher, as well as with higher total quantity of microorganisms during the phase of complete ripeness. Out of the fertilized variants the activity of the enzyme is highest at the fertilizing with ammonium nitrate, as well as with the using of a combined product urea+ammonium nitrate, and it is lowest with the fertilizing with UAN, but not lower than with the controls. This tendency correlates with the quantity of the microorganisms.

Regarding the seasonal dynamics the cellulase activity is higher during the spring and autumn in comparison with the summer.

As it has been indicated the activity of the studied enzymes depends on a number of factors. Regarding the reserve with nutritive elements Xiao et al. (2015) have determined, that the cellulase activity displays bigger dependency on the content of carbon, nitrogen, phosphorus and potassium in the soil, while the correlation between the catalase and phosphorus and potassium is significantly weaker.

According to Balezentiene and Klimas (2009) studies the activity of some enzymes (urease and sucrase) is higher in the beginning of the vegetation (spring) than during the autumn and reaches a peak value in the middle of the summer because of the unused resources of the substrata in the soils and increase of the temperature and moisture. At the present studies the increase of the temperature and moisture of the soils during the spring contributes for increase of the activity of the catalase and cellulase, and during the summer the enzymes are suppressed by the drying and the accumulated unused substrata.

Conclusion:-

1. The fertilizing with the used fertilizer products increases the biogenity of the soils – the total microflora is with higher values at the fertilized soil samples in comparison with the controls (except for the fertilizing with UAN in phases tillering and ripening, as well as with the fertilizing with ammonium nitrate in phase tillering). At the present experiment it is highest with the combined usage of urea and ammonium nitrate (two times in comparison with the control), followed by the fertilizing only with ammonium nitrate and the total quantity of the microorganisms is lowest with the usage of the fertilizer UAN.
2. The ammonifying microorganisms (non-spore-forming bacteria and bacilli) occupy highest percentage share in the composition of the total microflora with all soil samples – around 90%. At the phase of complete ripeness a higher percentage share occupy the bacilli, which are spore-forming and can survive better unfavourable conditions. Largest difference between the percentages of the bacilli and the non-spore-forming bacteria in favour of the first group– 44,2 % is determined namely with the fertilizing with UAN, where the total quantity of the microorganisms is lowest.
3. The actinomycetes are weaker presented in the composition of the total microflora (after the ammonifiers), which quantity in comparison with the control is increasing with the combined fertilizing with urea and ammonium nitrate.
4. The micromycetes occupy lowest percentage share – around 1-5 % of the composition of the total microflora. Their quantity is higher with the fertilized soils in comparison with the control. Consequently the development of the micromycetes depends also on a complex of factors – moisture, temperature of the soil, pH, reserve with nutritive substances, type of vegetation.
5. The quantity of the bacteria, assimilating mineral nitrogen is highest with the control in first and third phase, and with the combined fertilizing with urea and ammonium nitrate at the phase of complete ripeness, and lowest with the fertilizing only with ammonium nitrate at the same phase and at the usage of UAN for the other two phases.
6. Regarding the seasonal dynamics the quantity of the microorganisms is highest during the summer. Obviously a complex of factors influences their development and activity – moisture, temperature, fertilizing (the content of the fertilizer, the norm of fertilizing, the form of the elements carried in), reserve with nutritive substances, pH, mechanical composition of the soil, type of vegetation and other factors.
7. A higher catalytic activity is determined with the controls and combined fertilizing with urea and ammonium nitrate, and the activity of the enzyme is lowest with the fertilizing with UAN, which correlates also with the

- lower quantity of microorganisms with this variant. Regarding the catalytic activity studied in seasonal dynamics the values of the enzyme are higher during the spring in comparison with summer and autumn.
8. The fertilizing increases the cellulase activity. Out of the fertilized variants the enzyme activity is highest with the fertilizing with ammonium nitrate, as well as with the usage of a combined product urea+ammonium nitrate, and lowest with the fertilizing with UAN, but not lower than with the controls. Regarding the seasonal dynamics the cellulase activity is higher during the spring and autumn in comparison with the summer for both of the studied crops.
 9. The analyzed microbiological and enzyme indexes can serve as sensitive biological and biochemical indicators at fertilized soils with purpose determining best variants of fertilizing for increasing the soil fertility and obtaining qualitative high-yield agricultural produce.

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