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INFLUENCE OF HEAVY METALS ON MICROORGANISMS TAKING PART IN THE CIRCULATION OF NITROGEN IN SOIL

WPŁYW METALI CIĘŻKICH NA MIKROORGANIZMY UCZESTNICZĄCE W OBIEGU AZOTU W GLEBIE

Abstract: Nowadays natural cycles of nitrogen have undergone essential changes. The number of heavy metals polluting soil possess an oligodynamic effect (bactericidal ability) which may affect the process of circulation of biophilic elements, first of all of nitrogen, appreciably. The programme of researches included microbiological investigations in a model experiment with sod-podzol soil polluted with heavy metals. The results of the model experiment showed that increasing of soil pollution with heavy metals led to decrease of the quantity of *Azotobacter* and decrease of *Clostridium* bacteria's ability for butyric fermentation till its full loss, to inhibit of vital functions of ammonificating bacteria.

Keywords: heavy metals, soil, landscape, microorganisms, nitrogen

Microorganisms are elementary components of the biosphere. They are necessary links in food chains because transformation of important chemical elements on the Earth is carried on by biochemical activity of microorganisms. With the help of bacteria the circulation of biogenic elements is carried on including nitrogen. Nitrogen is the most important chemical elements. It is part of proteins, nucleic acids and chlorophyll. Nitrogen compounds play an important role in the process of photosynthesis, metabolism, new cells' formation. Nitrogen is irreplaceable in the forming of top-soil and its fertility, in increasing of agriculture effectiveness. Anthropogenesis has affected natural

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processes of biological fixation and migration of nitrogen appreciably. Nowadays natural cycles of nitrogen have undergone essential changes.

On the one hand, intensification of farming leads to rapid decrease of humus and nitrogen stores in soil, on the other hand, the entrance of *heavy metals* (HM) in the environment has increased sharply which affects microorganisms taking part in transformation of nitrogen compounds [1].

The number of HM polluting soil possess an oligodynamic effect (bactericidal ability) which may affect the process of circulation of biophilic elements, first of all of nitrogen, appreciably [2, 3].

The problem of soil pollution with HM is of current importance for Ryazan region characterized by developed agriculture and intensive man-caused influence on the environment.

Materials and methods

Researches were conducted at an ecological range representing a large-scale model of a closed drained strip of Oka-river basin's left bank.

The programme of researches included microbiological investigations in a model experiment with sod-podzol soil polluted with heavy metals (HM). During the arrangement of a model experiment for coverage of the diapason of soil pollution from admissible to extremely dangerous 3 variants were worked out which were based on the *total index of Zc pollution* (its value corresponds to a certain category of pollution). The level of pollution covered the range from admissible to extremely dangerous. HM were introduced into soil in the following concentrations: 0.5 of their *approximate admissible concentration* (AAC) (1 variant), 1.5 of AAC (2 variant), 4.5 of AAC (3 variant) (Table 1).

Table 1

The scheme of the experiment

Metal	The variants of the experiment (the content of heavy metals in soil [mg · kg^{-1}])*			
	Control (the initial soil)	1 (0.5 of AAC)**	2 (1.5 of AAC)	3 (4.5 of AAC)
Cu	10.8	154.2	484.2	1474.2
Zn	123.5	151.5	701.5	2351.5
Pb	40.0	120.0	440.0	1400.0
Cd	0.4	2.1	7.1	22.1
Zc	1	247.5	802.6	2467.9
The category of pollution	1 admissable	2 reasonably dangerous	3 dangerous	4 extremely dangerous

* The content of HM in soil in variants is given without the background taken into account; ** ACC for sod--podzol soil.

In the variants of the experiment with different pollution levels the quantity of aerobic and anaerobic and ammonificating bacteria was identified by means of standard methods.

For revealing of *Azotobacter* the method of soil nubbs was used. Test soil was moistened with sterile water till a paste-like condition, then with the help of a bacterial loop it was put in Petri dishes (50 nubbs in each Petri dish) on Ashbi nutrient medium. The Petri dishes were incubated in a thermostat at 37 ^{o}C during 4–6 days. After incubating the quantity of the nubbs overgrown with mucous colonies of *Azotobacter* was counted, the percentage of overgrowing was calculated.

For revealing of anaerobic nitrogen-fixing bacteria of *Clostridium* in the soil under consideration the method of accumulative culture in liquid medium by Vinogradskij was used. This method is qualitative, the conclusion about vital functions of nitrogen--fixing bacteria of *Clostridium* was made on the ground of the visual signs of their growth. The nutrient medium was poured in test tubes with a high layer, then nubbs of the soil under consideration was sowed and incubated at 80 ^{o}C during 10 minutes for the purpose of removal of sporeless bacteria from the concomitant nitrogen-fixing ones. The crops were incubated in anaerobe conditions at 37 ^{o}C during 2–3 days. Butyric fermentation that appeared in elective conditions and followed by allocating of gas bubbles was the evidence of the evolution of anaerobe cryptogamic bacteria. Glucose that is a component of the Vinogradskij medium turns into butyric acid and carbonic acid as the result of the vital functions of the anaerobes, a lot of froth appears in the test tubes what was marked visually.

For revealing of ammonificating bacteria in the soil under consideration the method of sowing from soil suspensions on *meat peptone agar* (MPA). The culture of ammonificators was obtained by sowing of MPA soil nubbs in test tubes. Under the plugs of the test tubes litmus paper for revealing of ammonia and paper sodden with lead acetate for revealing of hydrogen sulphide were put. The crops were incubated in a thermostat at 30 ^{o}C during 2–3 days. This method is qualitative, the change of the colour of test paper defined visually was the evidence of vital functions of ammonificating bacteria [4].

Revealing of anaerobe nitrogen-fixing bacteria of *Clostridium* and ammonificating bacteria was held with the help of qualitative methods based only on visual methods fixed with survey gear. The obtained data do not have graphical or tabular presentation.

All the experiments had thrice-repeated replication, the obtained data in the experiment of revealing of *Azotobacter* were exposed to computer processing.

Results and discussion

Fertility and the ability of self-cleaning depends on microorganisms taking part in the transformation of nitrogen compounds.

An *Azotobacter* is a freely-moving nitrogen-fixing bacteria. An *Azotobacter* consumes ammonium salts, nitrates(V), nitrates(III) and amino acids as the sources of nitric nutrition. It is an active producer of bacteria, enriches soil with nitrogen, is capable of assimilation of compounds oxidizing with difficulty [5–7].

Ready nutrient medium was sowed with nubbs of the soil under consideration. Overgrown with phlegm nubbs was evidence of the presence of *Azotobacter* in soil. The results of the researches are given in Fig. 1.

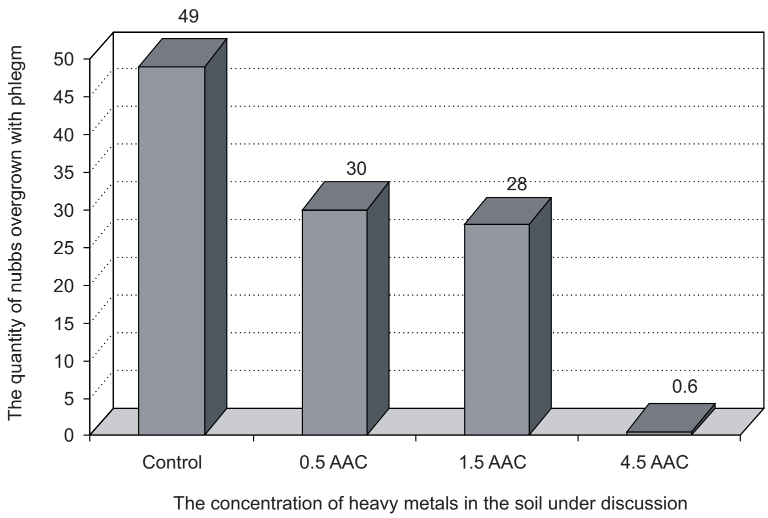

Fig. 1. The dependence of an *Azotobacter's* growth on the level of soil pollution with heavy metals

Consequently, decrease of the quantity of nubbs overgrown with phlegm is in inverse negative relationship with the level of its pollution with heavy metals which is proved by the value of the coefficient of correlation (r = –0.97). So the more concentration of metals in soil is, the less bacteria are. If the concentration is 4.5 of AAC, the *Azotobacter* is not practically identified. Consequently, such a concentration is destructive for an *Azotobacter*. *Clostridium pasteurianum* is a nitrogen-fixing anaerobe that connects molecular nitrogen only if there is no combined nitrogen [5, 8]. Test tubes with nutrient medium were sowed with nubbs of soil containing heavy metals in different concentrations (the control soil, 0.5 of AAC, 1.5 of AAC, 4.5 of AAC). The presence of *Clostridium pasteurianum* was judged by turbidity of the medium and allocation of gas bubbles that is formed while butyric fermentation. In the test tubes with the control soil on the second or the third day after the sowing the medium grew turbid and active allocation of gas bubbles was observed. This was evidence of the presence of *Clostridium pasteurianum.*

In the test tubes that were sowed with the soil with such concentrations of heavy metals as 0.5 AAC and 1.5 AAC the same processes were running and it also showed the presence of bacteria *Clostridium.* But alongside with it the decrease of the intensity of fermentation was noticed and it showed inhibition of a bacterium's activity by heavy metals. In the test tubes with the soil containing heavy metals in such a concentration as

4.5 from AAC the signs of butyric fermentation (turbidity of the medium, allocation of gas bubbles) were not noticed in all the three replications. The results of the researches are presented in the Fig. 2.

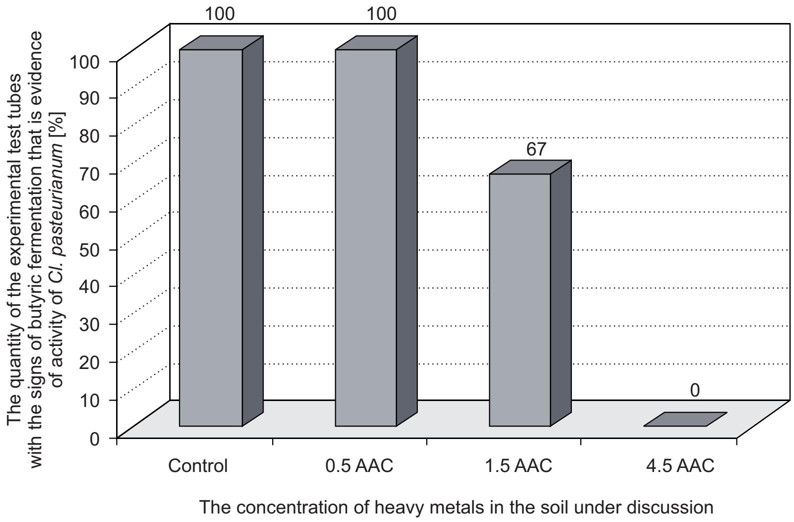

Fig. 2. Dependence of the intensity of butyric fermentation caused by *Cl. pasteurianum* on the level of soil pollution with heavy metals

There can be two reasons for it: pollution of soil with heavy metals blocked the ability of bacteria for fermentation; pollution of soil with heavy metals leads to death of *Clostridium pasteurianum*.

In the model experiment the influence of HM on ammonificating bacteria was studied. In the process of ammonification of proteins aerobes (*Bacillus mycoides*, *B. megatherium*, *B. mesentericus*, *B. subtilis*, *B. prodigiosum* and others), facultative anaerobes (*B. proteus vulgaris*, *E. coli*) and anaerobes (*Cl. putrificus*, *Cl. sporogenes*) took part [5, 7, 9].

The test tubes with nutrient medium were sowed with nubbs of soil polluted with heavy metals in different concentrations (control, 0.5 of AAC, 1.5 of AAC, 4.5 of AAC). Allocation of ammonia and hydrogen sulphide that were formed as a result of putrefaction of proteins indicated the presence of bacteria. In the results of the experiment it was found out that in the test tubes with the control soil and the soil with heavy metals in such concentrations as 0.5 of AAC and 1.5 of AAC ammonia and hydrogen sulphide allocated. Consequently, one can make a conclusion that ammonificating bacteria were present in the soil under consideration.

In the test tubes where the soil in the concentration 4.5 AAC was sowed gas did not allocate and it is evidence of death of ammonificating bacteria. In the test tubes where the soil with heavy metals in the concentration 4.5 AAC was sowed gas did not allocate and presumably it was evidence of death of ammonificating bacteria. The results of the researches are presented in the Fig. 3.

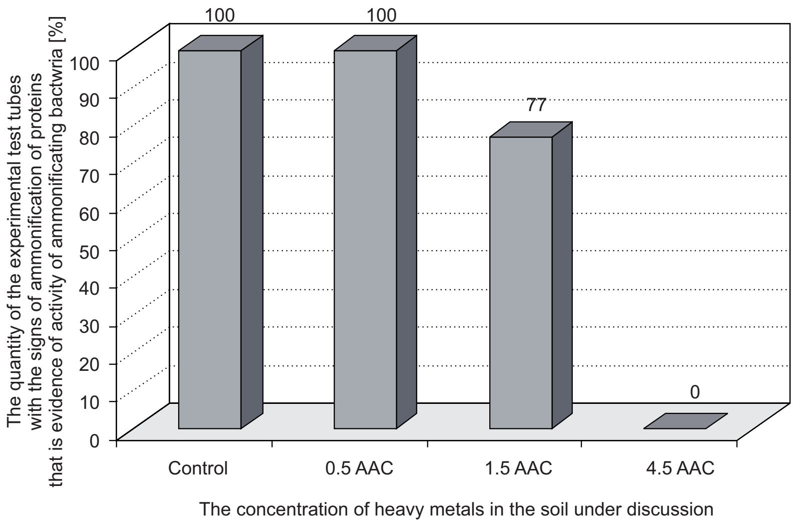

Fig. 3. The dependence of the intensity of the process of ammonification of proteins on the level of soil pollution with heavy metals

Conclusion

1. According to the results of a model experiment one can make a conclusion that decrease of soil pollution with heavy metals leads to the decrease of the quantity of *Azotobacter*.

2. The increase of the concentration of heavy metals in soil decreases the ability of bacteria of *Clostridium* for butyric-acid fermentation till its complete loss.

3. The increase of heavy metals (HM) in soil leads to inhibition of vital functions of ammonificating bacteria.

4. The results of the experiment carried out showed that if in sod-podzol soil of the landscape of Oka-river's left bank there are heavy metals in the amount exceeding AAC, there is a real danger of the violation of nitrogen circulation and of the decrease of soil fertility as a result.

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WPŁYW METALI CIĘŻKICH NA MIKROORGANIZMY UCZESTNICZĄCE W OBIEGU AZOTU W GLEBIE

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Abstrakt: W przyrodniczym obiegu azotu zaznaczyły się ostatnio istotne zmiany. Zanieczyszczenie gleby metalami ciężkimi powoduje działanie oligodynamiczne (zdolność bakteriobójczą), co może istotnie zachwiać procesy obiegu pierwiastków biogennych, w pierwszej kolejności azotu. Program badań obejmował mikrobiologiczne badania w eksperymencie modelowym z glebą darniowo-bielicową zanieczyszczoną metalami ciężkimi. Wyniki tego doświadczenia wykazały, że zwiększenie zanieczyszczenia gleby metalami ciężkimi prowadzi do zmniejszenia liczebności, bakterii z rodzaju *Azotobacter*. Ponadto w przypadku bakterii z rodzaju *Clostridium* wykazano obniżenie zdolności do przeprowadzenia fermentacji masłowej, aż do pełnej jej utraty. Jednocześnie odnotowano zahamowanie życiowych funkcji bakterii amonifikacyjnych.

Słowa kluczowe: metale ciężkie, gleba, krajobraz, mikroorganizmy, azot