UDC 631.46.631.445.41:631.84

https://doi.org/10.15407/biotech10.03.065

# EFFECT OF HEAVY METAL IONS ON THE NUMBER AND ACTIVITY OF Azotobacter AND MELANIN-SYNTHESIZING MICROMYCETES

I. M. Malynovska

National Scientific Center "Institute of Agriculture of the National Academy of Agrarian Sciences of Ukraine", Chabany, Ukraine

E-mail: irina.malinovskaya.1960@mail.ru

Received 26.12.2016

The aim of the work was to determine the possibility of using the number and activity of Azotobacter cells and melanin-synthesizing micromycetes as indicators of gray forest soils of different types (fallow, extensive and intensive agrosoil) pollution with heavy metal ions. For this purpose, there were used laboratory-analytical, microbiological and statistical methods. As a result of research of increasing doses of heavy metals (zinc + lead) influence on the number of microorganisms in the gray forest soils it was found that the number and activity of Azotobacter and the number and part of melanin-synthesizing micromycetes in their total number may be fit into indicators of pollution with heavy metals. Azotobacter cells activity index may be considered indicative at contamination levels of 5-100 of maximum permissible concentration in the absence of vegetation, at contamination levels of 10-100 — for soils with phytocenosis. The number and proportion of melanin-synthesizing micromycetes in total guantity may serve as diagnostic sign of gray forest soils pollution with high doses of heavy metals, but only for the period of contamination up to 2 years.

It was shown that nature of the effect of heavy metals on the number of microorganisms of indicative groups depended on the presence of plants in the monitoring system, on doses of heavy metals, on the term of contamination and on the type of soil usage.

Key words: Azotobacter, melanin-synthesizing micromycetes, diagnostic indicator, pollution, heavy metals.

The need to study the impact of heavy metals on soil microbial communities does not cause doubt because the microorganisms are the first in food chain for heavy metals entering into the organisms of higher animals and humans. In addition, with soil microorganisms usage the degree of soil contamination by heavy metals can be controlled, so microorganisms can be used as indicators of ecotopes pollution [1-3]. Evaluation parameters of the properties of microorganisms-indicators of soil pollution are divided into two main groups. Firstly, there are parameters of the total activity of microbial community: intensity of carbon dioxide respiration, activity of mineralization nitrogen compounds immobilization, certain enzymes activity, and so on. Secondly, there are indicators of microorganisms of certain ecological-trophic, functional and taxonomic groups number, indicators of microbial and fungal biomass amount. This parameters combination is possible with the simultaneous use of data on the abundance and activity of microorganisms-components of microbial communities.

The aim of our study was to determine the possibility of microorganisms such as *Azotobacter* and melanin-synthesizing micromycetes usage as indicators of gray forest soils of different types pollution level with heavy metal ions.

# **Materials and Methods**

Model experiment was conducted using gray forest soils of stationary experiment of the laboratory of cereals and maize intensive technologies of National Scientific Center "Institute of Agriculture of the National Academy of Agrarian Sciences of Ukraine" (experimental farm "Chabany" Kyiv-Svyatoshinsky district, Kyiv region): extensive agrosoil — field crop rotation without the use of mineral and organic fertilizers

from 1987; intensive agrosoil — field crop rotation with fertilizers N<sub>96</sub>P<sub>108</sub>K<sub>112.5</sub> saturation backgrounded by plowing crop byproducts. The 0-20 cm layer of extensive soil variant contained: humus -1.31%, alkaline hydrolyzed nitrogen — 6.44 mg, nitrate nitrogen - 0.45, ammonium nitrogen — 0.18, mobile phosphorus — 22.5and exchangeable potassium — 5.90 mg per 100 g of dry soil; phosphorus level of mobility — 0.21 mg  $P_2O_5/100$  g of the soil,  $\mathrm{pH}_{\mathrm{(KCl)}}$  — 5.7. The 0 — 20 cm layer of intensive soil variant contained: humus -1.75%, alkaline hydrolyzed nitrogen - 6.86 mg, nitrate nitrogen - 6.46, ammonium nitrogen — 0.20, mobile phosphorus — 60.0and exchangeable potassium — 25.4 mg per 100 g of dry soil; phosphorus level of mobility —  $0.66 \text{ mg P}_2\text{O}_5/100 \text{ g of the soil}$ , pH<sub>(KCl)</sub> — 4.9. Fallow soil was characterized by the following indexes: humus -2.74%alkaline hydrolyzed nitrogen — 9,33 mg, mobile phosphorus —  $36.8\,$  mg and exchangeable potassium — 15.3 mg per 100 gof dry soil,  $pH_{(KCl)} - 5.6$ .

The soil was taken in the fall and before the experiment its biological activity was restored by moisturizing and thermostatting at 25 °C for 21 days. We investigated the variants with artificially created backgrounds of zinc and lead: 1, 2 — soil with natural concentrations of heavy metals with and without phytocenosis (corn); 3, 4 — exceeding the maximum permissible concentration (MPC) 5 times; 5, 6 — exceeding the MPC 10 times; 7, 8 – exceeding the MPC 100 times. When creating contamination backgrounds, we took into account the acid soluble metal fraction, since it is considered the main technogenic part of the stock of heavy metals in soil. For 8 days before the introduction of heavy metals, in some part of vessels we sowed corn seeds. Into control vessel to equalize the nitrogen content, we add KNO<sub>3</sub> solution of appropriate concentrations.

Microbiocenosis state was examined at 1, 21, 31 days, 6, 12, 18 and 24 months after the introduction of heavy metals. The number and activity of microorganisms of major ecological-trophic groups, microbiological processes targeting were determined by methods that are described earlier [4]. Statistical analysis of the results was performed using modern computer software package *Microsoft Office*.

## **Results and Discussion**

The results shown in the Table 1 indicate the complexity of the nature of the heavy

metals effect on the number of *Azotobacter*: it depends on the presence of plants in the system, the dose of heavy metals, the incubation period of contaminated soil and the type of its previous usage. Thus, in the soil with natural content of heavy metals (control) the plants perform protective function for Azotobacter under intensive use of soil in agricultural production and in the long fallow state. Under the extensive use of gray forest soil in small terms of pollution the number of Azotobacter in variant with phytocenosis is lower compared to the variant without plants, and after 6 months of incubation the difference between the variants is unreliable. Especially noticeable difference between the types of soil is found under the conditions of pollution with heavy metals: in fallow soil at pollution with heavy metals at a dose of 5 MPC the Azotobacter number in the variant with phytocenosis exceeds its amount in the variant without plants at incubation period for 1 day 1.69 times, 32 days — 9 070 times, in intense agrosoil the corresponding indexes are 1.66 and 1.41 times. In extensive agrosoil, the protective action of plants on *Azotobacter* population in the respective variants of the experiment at 5 MPC and greater levels of contamination were not observed.

Previous studies of microbial communities structure of gray forest soils with differently targeted usage has shown that by targeting and intensity of mineralization processes the intensive agrosoil is more like a fallow soil, because at this applying method, large or 100 % part of biomass, which has grown in this soil, returns to the soil and along with it — macro- and microelements [4]. At intensive usage, the exogenous sources of mineral elements are applied in a soil and crop by-products are turned into the soil, which leads to profound differences from extensive agrosoil, which alienates macro-, microelements and carbon for 24 years.

On the first day of observations in fallow soil at small contamination levels, the plants perform protective function for *Azotobacter*, and at high contamination levels, they cannot do it through biochemical stress. However, after 32 days the protective function of the plants is manifested in all variants except variant with a maximum contamination level (100 MPC). In intensive agrosoil, the protective function of plants is observed at all terms of observations and at all contamination levels except the maximal (Table 1). A possible reason for these differences between the variants of gray forest soils usage may be the

Table 1. Azotobacter amount in gray forest soils of different usage types depending on the term of pollution with heavy metals (% of mud balls overgrowing)

Variant	Fallow		Intensive agrosoil		Extensive agrosoil						
	1 day	32 days	1 day	32 days	1 day	21 days	6 months	12 months	18 months	24 months	
Control with- out plants	$34.8 \pm \\2.11$	$\begin{array}{c} 0.67 \pm \\ 0.04 \end{array}$	58.7 ± 5.88	$60.0 \pm \\ 4.55$	$100.0 \\ \pm 10.5$	100.0 ± 11.2	$98.7 \pm \\10.5$	$\begin{array}{c} 93.3 \pm \\ 7.72 \end{array}$	$\begin{array}{c} 95.3 \pm \\ 9.01 \end{array}$	86.0 ± 6.88	
Control + phytocenosis	$37.4 \pm \\ 2.01$	$\begin{array}{c} 4.00 \pm \\ 0.54 \end{array}$	94.0 ± 9.01	$62.0 \pm \\ 4.15$	$94.7 \pm \\ 8.74$	$90.7 \pm \\10.5$	$97.3 \pm \\11.2$	$\begin{array}{c} 92.0 \pm \\ 7.44 \end{array}$	88.7 ± 7.88	90.7 ± 9.28	
5 MPC with- out plants	19.6 ± 1.88*	0.01 ± 0.001*	$45.3 \pm 2.02*$	$22.7 \pm \\ 2.45 *$	96.0 ± 9.88	98.0 ± 8.98	$98.7 \pm \\ 8.56$	$95.3 \pm \\ 8.24$	88.0 ± 7.82	$92.7 \pm \\10.5$	
5 MPC + phy- tocenosis	$\begin{array}{c} 33.2 \pm \\ 2.04 \end{array}$	90.7 ± 8.75*	$75.3 \pm 6.22*$	31.0 ± 2.88*	$96.0 \pm \\10.5$	98.2 ± 8.74	99.0 ± .99	100 ± 9.46	$\begin{array}{c} 95.3 \pm \\ 9.22 \end{array}$	93.3 ± 8.92	
10 MPC with- out plants	$20.4 \pm \\1.69*$	$0.67 \pm 0.071$	0.07 ± 0.006*	9.00 ± 0.88*	$\begin{array}{c} 92.0 \pm \\ 10.6 \end{array}$	$\begin{array}{c} 98.7 \pm \\ 8.76 \end{array}$	$96.7 \pm \\ 9.55$	$\begin{array}{c} 98.7 \pm \\ 6.44 \end{array}$	$\begin{array}{c} 97.3 \pm \\ 8.91 \end{array}$	86.0 ± 8.27	
10 MPC + phytocenosis	$14.4 \pm \\1.22*$	10.0 ± 0.98*	$30.7 \pm 2.12*$	17.6 ± 0.168*	$\begin{array}{c} 97.3 \pm \\ 9.98 \end{array}$	$\begin{array}{c} 97.3 \pm \\ 10.1 \end{array}$	$100.0 \pm \\ 12.0$	100.0 ± 9.19	100.0± 8.58	100.0 ± 10.8	
100 MPC without plants	17.4 ± 1.58*	20.0 ± 1.68*	0.67 ± 0.054*	0*	0*	0*	0*	0*	0*	0*	
100 MPC + phytocenosis	12.0 ± 1.09*	$\begin{array}{c} 4.67 \pm \\ 0.55 \end{array}$	0*	0*	0*	0*	0*	0*	0*	0*	

*Note:* hereinafter \* — P < 0.05 (compared to control).

different levels of anthropogenic pressure, which in intensive agrosoil is the highest among the studied variants; and counteraction mechanisms to the stressors of chemical type, which include heavy metals, in this variant are activated.

Azotobacter belongs to an important group of microorganisms that are indicators of ecological purity of the soil and its quantity decreases when introducing many pollutants, including petroleum products [5-7]. This is confirmed by experimental data on the number of Azotobacter reduction through one day after entering the heavy metals. In the fallow soil it is reduced at 5 MPC doses of heavy metals in variant without plants by 77.6%, in the root zone of phytocenosis — by 4.82%; at 10 MPC doses of heavy metals the Azotobacter number is reduced respectively by 70.6 and 159.7%; at 100 MPC — by 100.0 and 211.7%. Consequently, at small pollution levels the Azotobacter number decreases slower in root zone of phytocenosis, and at 10 and 100 MPC — in the absence of phytocenosis.

The protective function of plants for *Azotobacter* also is shown by the results of studying the activity of these microorganisms

by Kozhevin and colleagues method [8], which allows determining the number and activity of Azotobacter cells in soil simultaneously in the analysis of the emergence of bacterial colonies on the nutrient medium. Analysis of microbial cells activity is based on the assumption that the probability of colonies formation in a laboratory depends on the parental cell state in nature. According to the analysis, the probability of colonies formation (PCF) of Azotobacter in the rhizosphere of plants is higher than the similar indicator of soil without plants, in control -2.94 times, at 5 MPC — 195 times, at 10 MPC indices have the same value, at the maximal pollution of soil with heavy metals the Azotobacter cell activity, as well as its quantity, is the maximal in soil without plants (Table 2). Experimental data on changes of Azotobacter physiological and biochemical activity in contaminated soil also confirmed the possibility of its use as a diagnostic microorganism for heavy metals pollution. Thus, PCF of Azotobacter in the uncontaminated soil is 72 times higher than indexes of variants with pollution of 5 and 10 MPC, 2.18 times — of 100 MPC (without phytocenosis), in variants with phytocenosis

№	Variant	Ammonifiers	Mineral nitrogen immobilizers	Oligonitrophiles	Denitrifiers	Micromycetes	Azotobacter	
1	Control without plants	1.50 ± 0.08	$\begin{array}{c} 0.158 \pm \\ 0.012 \end{array}$	$5.56 \pm 0.45$	$0.836 \pm 0.07$	$1.69 \pm 0.14$	$0.723 \pm 0.08$	
2	Control + phy- tocenosis	$0.367 \pm 0.021$	$0.153 \pm 0.011$	$3.11 \pm 0.25$	$13.5 \pm 0.11$	$2.98 \pm 0.22$	$2.12 \pm 0.09$	
3	5 MPC without plants	$0.424 \pm 0.052*$	$0.189 \pm 0.017$ *	$1.02 \pm 0.09*$	$0.439 \pm 0.04$ *	$2.13 \pm 0.15*$	$0.010 \pm 0.001$ *	
4	5 MPC + phyto- cenosis	$1.88 \pm 0.150 *$	$0.146 \pm 0.011$	$2.78 \pm 0.19$	$10.7 \pm 0.95$ *	$1.06 \pm 0.09*$	$1.95 \pm 0.21$	
5	10 MPC without plants	$1.53 \pm 0.131$	$0.054 \pm 0.004$ *	$2.46 \pm 0.15*$	$0.192 \pm 0.02*$	$0.556 \pm 0.06*$	$0.010 \pm 0.001$ *	
6	10 MPC + phy- tocenosis	$2.47 \pm 0.189*$	$0.065 \pm 0.005$ *	$2.83 \pm 0.21$	$13.0 \pm 1.14$	$2.24 \pm 0.28*$	$0.010 \pm 0.001$ *	
7	100 MPC with- out plants	$0.971 \pm 0.085*$	$0.063 \pm 0.007$ *	$1.24 \pm 0.13*$	$4.01 \pm 0.52*$	$5.41 \pm 0.45*$	$0.332 \pm 0.04*$	

 $2.73 \pm 0.180 * |0.017 \pm 0.002 * |3.80 \pm 0.22 * |0.294 \pm 0.02 * |2.21 \pm 0.18 *$ 

Table 2. Probability of colonies formation of nitrogen cycle microorganisms  $(\lambda, h^{-1} \cdot 10^{-2})$  in gray forest soil (fallow) contaminated with heavy metals for 32 days

corresponding figures were 1.09, 212 and 15.8. Thus, soil pollution with heavy metal ions inhibits Azotobacter cells activity, and this figure can be considered indicative for pollution of gray forest soils at contamination levels of 5–100 MPC in the absence of vegetation, at the pollution level of 10–100 MPC — on soils with phytocenosis. The reason of Azotobacter special sensitivity to the toxic effects of heavy metals may be the fact that the heavy metal ions cause in this microorganism simultaneously the protein, RNA and DNA synthesis inhibition [9], whereas in other microorganisms — mainly protein or RNA synthesis [10].

100 MPC + phy

tocenosis

At intensive agrosoil incubation for 32 days, the pattern becomes noticeable: the higher the level of soil pollution with heavy metal ions, the more pronounced is the protective function of plants for Azotobacter. In particular, the number of Azotobacter in the rhizosphere of plants is higher than the indexes of soil without plants, in control by 3.33%, at 5 MPC — by 36.6, at 10 MPC by 95.6% (Table 1). During this incubation period, the number of Azotobacter decreases with the doses of pollutant increasing in soil without plants: at 5 MPC -2.64 times, at 10 MPC - 6.67 times; the corresponding figures for the rhizosphere of plants are 2.0 and 3.52 times. For the maximal level of soil pollution with heavy metals (100 MPC), the mud balls overgrowing method do not detect the *Azotobacter*. Thus, a series of model experiments shows that the number and activity of *Azotobacter* cells are diagnostic indicators of the intensity of gray forest soil pollution with heavy metal ions.

 $0.134 \pm 0.02*$ 

The ability to form melanoid pigments is believed the protective reaction of microorganisms to anthropogenic pollution [11]. The results of our modeling studies confirm this finding: the pollution with heavy metal ions in a day leads to an intensification of the synthesis of melanin-like pigments and increase in the number of melaninsynthesizing micromycetes at 5 MPC 4.40 times, 10 MPC - 5.90, 100 MPC - 3.75 times (Table 3). At the root zone of plants, the number of melanin-forming micromycetes almost independent of pollutant dose, except the variant with 100 MPC, where an increase 1.69 times in the number of CFU is observed. At pollution levels of 5-10 MPC the plants in the first stage perform a protective function for microorganisms of own rhizosphere, so the latter do not need to synthesize melanoid

With pollution level increasing, not only the number of melanin-synthesizing micromycetes, but their part in the total number of micromycetes, especially at 100 MPC, significantly increases. On the 21<sup>st</sup> day of incubation, the situation in the root zone of plants varies: the number of melanin-synthesizing micromycetes increases

 $Table \ 3.$  Influence of pollution period with heavy metal ions on the total number of micromycetes and their melanin-synthesizing forms in gray forest soil (extensive agrosoil)

		%	14.1	20.6	52.3	21.2	24.3	11.5	1.80	2.33
	24 months	2	$\begin{array}{c} 4.99 \pm \\ 0.51 \end{array}$	$9.96 \pm 0.87$	$5.23\pm\\0.44$	$10.8 \pm 0.82$	$6.59 \pm 0.42 *$	$\begin{array}{l} 4.56 \pm \\ 0.22 * \end{array}$	$2.23 \pm 0.12 *$	$2.25 \pm 0.15 ^{*}$
		1	35.3 ± 2.88	$48.3 \pm 3.16$	$34.4 \pm \\ 2.88$	$51.0 \pm 4.17$	27.1 ± 2.11*	$39.6 \pm \\ 2.89 *$	123.3 ± 9.87*	96.5 ± 8.45*
	18 months	%	33.6	30.7	40.4	29.5	36.8	48.5	7.65	27.0
		2	4.20 ± 0.28	5.00 ± 0.45	$5.50\pm\\0.48*$	4.30 ± 0.29	5.01 ± 0.45*	4.92 ± 0.36	5.72 ± 0.24*	32.3 ± 0.45*
		1	$12.5 \pm 1.08$	$16.3 \pm 1.44$	$13.5 \pm 1.13$	$14.6 \pm 1.54$	$13.6 \pm 1.12$	$10.1 \pm 0.92 *$	74.5 ± 5.42*	119.6 ± 10.5*
	12 months	%	23.8	56.0	44.4	35.7	35.4	12.5	6.98	43.8
		2	$5.43\pm\\0.44$	5.10 ± 0.28	$\begin{array}{c} 4.35 \pm \\ 0.34 \end{array}$	$5.00 \pm 0.42$	$\begin{array}{l} 4.42 \pm \\ 0.38 \end{array}$	$1.10 \pm 0.05 *$	$3.31 \pm 0.13 ^{*}$	$16.9 \pm 0.94 *$
Incubation period		1	$22.8 \pm 0.26$	9.11 ± 0.84	9.80± 0.62*	$14.4 \pm 1.02 *$	12.5 ± 0.88*	8.80 ± 0.54	$47.4 \pm \\2.95 *$	38.6 ± 2.84*
cubati	6 months	%	28.1	36.0	38.0	47.8	61.4	62.5	58.3	56.0
l II		2	$3.65 \pm 0.28$	5.79 ± 0.56	$3.68\pm0.22$	$4.17 \pm 0.55 *$	6.14 ± 0.71*	5.56 ± 0.62	$18.5 \pm 1.22 *$	17.5 ± 1.08*
		1	$13.0 \pm 0.99$	$16.9 \pm 1.89$	$9.68\pm\\1.05*$	8.72 ± 0.88*	10.0 ± 0.99*	8.89 ± 0.94*	$31.7 \pm 1.22 *$	$31.2 \pm 2.48 \%$
	21 days	%	21.4	35.7	20.7	29.2	20.3	29.0	47.8	28.9
		2	$\begin{array}{c} 4.32 \pm \\ 0.55 \end{array}$	$6.03 \pm 0.44$	$\substack{7.25\pm\\0.65*}$	$9.41 \pm \\ 0.85 *$	5.84 ± 0.47*	$14.1 \pm 1.04 *$	8.07 ± 0.74*	$7.26 \pm 0.56 \pm$
		1	$20.2 \pm 1.15$	$16.9 \pm 1.02$	$35.1 \pm \\ 2.04 *$	$32.2 \pm \\ 3.85 *$	$28.8 \pm \\ 2.56 *$	$48.6 \pm 3.85 \%$	$16.9 \pm 0.84 \%$	$25.1 \pm 0.25 \%$
	1 day	** %	19.5	23.0	67.5	43.5	63.2	40.3	99.3	95.2
		2	$2.14 \pm 0.08$	$3.48\pm\\0.28$	$8.78\pm0.06*$	$\begin{array}{c} 3.62 \pm \\ 0.28 \end{array}$	$11.5 \pm 0.88 *$	$4.11 \pm 0.28 *$	7.60 ± 0.65*	$5.92 \pm 0.44$
		1	11.0 ± 0.81	$15.1 \pm 1.02$	$13.0\pm\\1.05*$	8.33 ± 0.85	$18.2 \pm 0.75 *$	$10.2 \pm 0.94 *$	$7.65 \pm 0.54 *$	$6.22 \pm 0.52 \times$
	Variant		Control without plants	Control + phytocenosis	5 MPC with- out plants	$5~\mathrm{MPC} + \mathrm{phy}$ - tocenosis	10 MPC with- out plants	10 MPC + phytocenosis	100 MPC without plants	100 MPC + phytocenosis

Note: 1— the total number of micromycetes,  $10^4$  CFU/g of absolutely dry soil; 2— the number of melanin-synthesizing micromycetes,  $10^4$  CFU/g of absolutely dry soil; \*\*— the part of melanin-synthesizing forms in total number of micromycetes (%).

depending on the dose of heavy metals: at 5 MPC - 1.56 times, at 10 MPC - 2.33times, at  $100 \, \text{MAC} - 1.20 \, \text{times}$ . Soil variants without phytocenosis are also characterized by increased number of melanin-synthesizing micromycetes compared with uncontaminated soil. At this, the part of melanin-synthesizing micromycetes practically does not change depending on the dose of heavy metals (with the exception of 100 MPC). After 6 months of contaminated soil incubation, the amount of melanin-synthesizing micromycetes ceases to depend on the dose of pollutant in the variant with phytocenosis at concentrations of heavy metals of 100 MPC, without phytocenosis — at 10 and 100 MPC. At 18 months incubation, this relationship is lost for all experimental variants except variant of phytocenosis with pollution of 100 MPC. At 24 months incubation of contaminated soil, the variants with pollution of 100 MPC are characterized by less number of melaninsynthesizing micromycetes than soils with lower pollution level. At this, the total number of micromycetes in variants with maximal pollution level increases sharply, indicating that at prolonged pollution of gray forest soil (extensive agrosoil) the other mechanisms to protect micromycetes cells

### REFERENCES

- 1. Bernard L., Maron P., Mougel C., Nowak V., Lévêque J., Marol C., Balesdent J., Gibiat J., Ranjard L. Contamination of soil by copper affects the dynamics, diversity, and activity of soil bacterial communities involved in wheat decomposition and carbon storage. Appl. Environ. Microbiol. 2009, 75 (23), 7565–7569.
- 2. Anderson J., Hooper M., Zak J., Cox S. Characterization of the structural and functional diversity of indigenous soil microbial communities in smelter-impacted and nonimpacted soils. Environ. Toxicol. Chem. 2009, 28 (3), 534-541.
- 3. Mosina L. V. Features of microbial components functioning in a soil-plant system in conditions of high soil contamination with heavy metals and unregulated recreation. *Proc. TSKhA*. 2010, N 282, p.1, P. 732–739. (In Russian).
- 4. Malinovska I. M., Dombrovska I. V. State of microbiota gray forest soils under different using. Visnyk Kyivskoho Natsionalnoho Universytetu. Ser. Biol. 2011, Issue 57, P. 21–25. (In Ukrainian).
- Selivanovskaya S. Yu., Kiyamova S. N., Latypova V. Z., Alimova F. K. Effect of sedimentary waste water containing metals on microbial cenosis of wood gray soil.

from the damaging action of heavy metals start to operate. Thus, the number and the part of melanin-synthesizing micromycetes in total micromycetes number may serve as a diagnostic sign of pollution with high doses of heavy metals only for contamination periods not exceeding 2 years.

Thus, as a result of work performance with increasing doses of heavy metals (zinc + lead) it is found that indicative indexes of gray forest soil pollution with heavy metals is the number and activity of Azotobacter cells, and the number and part of melaninsynthesizing micromycetes in total. The activity of *Azotobacter* cells can be used as an indicator at pollution with heavy metals level of 5-100 MPC in the absence of vegetation, at the pollution level of 10-100 MPC — on soils with phytocenosis. It is found that after 2 years of gray forest soil pollution with high doses of heavy metals the defense mechanism of micromycetes cells changes. They lose the ability to synthesize melanin-like pigments, so the number and the part of melaninsynthesizing micromycetes in the total number may be diagnostic sign of pollution with heavy metal ions only at pollution period not over 2 years.

- *Pochvovedeniye.* 2001, N 5, P. 588–594. (In Russian).
- 6. Malinovska I. M., Zinoviev N. A. The direction and intensity of microbiological processes in dark gray ashed soils contaminated with petroleum. Visnyk Poltavskoi derzhavnoi ahrarnoi akademii. 2010, N 4, P. 17-23. (In Ukrainian).
- 7. Kolosvary I. Data concerning the possibility of using the abundance of the Azotobacter cells as bioindicator of soil pollution. Stud. Univ. Babes-Bolyai. Biol. 1998, N 1-2, P. 137-141.
- 8. Kozhevin P. A., Kozhevina L. S., Bolotina I. N. Definition of bacteria state in ground. Doklady AN SSR. 1987, 297 (5), 1247–1249. (In Russian).
- 9. Bernlohr R. W., Webster G. C. Effect of chloramphenicol on protein and nucleic acid metabolism in Azotobacter agilis. J. Bacteriol. 1958, 76 (3), 233-238.
- 9. Khovrychev M. P., Semenova A. M., Rabotnova I. L. The action of zinc ions on Candida utilis. Mikrobiol. 1980, T. XIX, V. 1, P. 59-63. (In Russian).
- 10. Zhdanova N. N., Wasilewskaia A. N. Mushrooms containing melanin in extreme conditions. Kyiv: Naukova dumka. 1988, 196 p. (In Ukranian).

# ВПЛИВ ЗАБРУДНЕННЯ ІОНАМИ ВАЖКИХ МЕТАЛІВ НА ЧИСЕЛЬНІСТЬ ТА АКТИВНІСТЬ АЗОТОБАКТЕРА І МЕЛАНІНСИНТЕЗУВАЛЬНИХ МІКРОМІЦЕТІВ

І. М. Малиновська

ННЦ «Інститут землеробства НААНУ», Чабани, Україна

E-mail: irina.malinovskaya.1960@ mail.ru

Метою роботи було встановити можливість використання чисельності та активності клітин азотобактера і меланінсинтезувальних мікроміцетів як індикаторних показників рівня забруднення іонами важких металів сірого лісового ґрунту різних типів (переліг, екстенсивний та інтенсивний агроземи). Для виконання цього завдання було застосовано лабораторно-аналітичний, мікробіологічний і статистичний методи. У результаті дослідження впливу зростаючих доз важких металів (цинк + свинець) на чисельність мікроорганізмів у сірому лісовому ґрунті встановлено, що до індикаційних показників забруднення важкими металами можна віднести чисельність та активність азотобактера, а також чисельність та частку меланінсинтезувальних мікроміцетів у їх загальній кількості. Показник активності клітин азотобактера можна вважати індикаційним за рівнів забруднення 5-100 гранично допустимих концентрацій у разі відсутності рослинного покриву, за рівнів забруднення 10-100 — на ґрунтах із фітоценозом. Кількість та частка меланінсинтезувальних мікроміцетів у загальній кількості можуть слугувати діагностичною ознакою забруднення високими дозами важких металів сірого лісового ґрунту, однак лише за терміну забруднення не більше 2 років.

Показано, що характер впливу важких металів на чисельність мікроорганізмів індикаційних груп залежить від наявності рослин у системі моніторингу, дози важких металів, терміну забруднення і типу використання ґрунту.

*Ключові слова*: азотобактер, меланінсинтезувальні мікроміцети, діагностичний показник, забруднення, важкі метали.

# ВЛИЯНИЕ ЗАГРЯЗНЕНИЯ ИОНАМИ ТЯЖЕЛЫХ МЕТАЛЛОВ НА ЧИСЛЕННОСТЬ И АКТИВНОСТЬ АЗОТОБАКТЕРА И МЕЛАНИНСИНТЕЗИРУЮЩИХ МИКРОМИЦЕТОВ

И. М. Малиновская

ННЦ «Институт земледелия НААНУ», Чабаны, Украина

E-mail: irina.malinovskaya.1960@ mail.ru

Целью работы было изучение возможности использования численности и активности клеток азотобактера и меланинсинтезирующих микромицетов как индикаторных показателей уровня загрязнения тяжелыми металлами серой лесной почвы разных типов (залежь, интенсивный и экстенсивный агроземы). Для решения этой задачи были использованы лабораторно-аналитический, микробиологический и статистический методы. В результате изучения влияния возрастающих доз тяжелых металлов (цинк + свинец) на численность микроорганизмов в серой лесной почве установлено, что к индикаторным показателям загрязнения тяжелыми металлами можно отнести численность и активность клеток азотобактера, а также численность и долю меланинсинтезирующих микромицетов в их общем количестве. Показатель активности клеток азотобактера можно считать индикаторным при уровнях загрязнения 5-100 предельно допустимых концентраций в отсутствие фитоценоза, при уровнях загрязнения 10-100 — на почвах с фитоценозом. Количество и доля меланинсинтезирующих микромицетов в общем количестве могут служить диагностическим показателем загрязнения высокими дозами тяжелых металлов серой лесной почвы, однако только при сроках загрязнения, не превышающих 2 лет.

Показано, что характер влияния тяжелых металлов на численность микроорганизмов зависит от наличия растений в системе мониторинга, дозы тяжелых металлов, срока загрязнения и типа использования почвы.

**Ключевые слова:** азотобактер, меланинсинтезирующие микромицеты, диагностический показатель, загрязнение, тяжелые металлы.