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Microbial Monitoring of Heavy Metals Polluted Soils and Their Sanitation Technology

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Abstract: The hierarchic system of microbial characteristics for monitoring of heavy metals polluted soils is presented in this paper. It is based on microorganisms which are sensitive to heavy metals and their physiological and biochemical activity at various level of its organization: precell, cell, population and cenosis. Besides this some meliorantes (meliorates) were tested as the way for reabilitation (rehabilitation) polluted soil.

Key words: microbial monitoring, hierarchic system, soil, microorganisms, meliorate

Introduction

Investigation the effect of specific anthropogenic factors, such as heavy metals, on microbial community function is necessary far elaboration of scientific foundation of rational soil using and environment protection. Besides this obtained data will permit to change and improve separate technologies and work out the ways of environment sanitation.

Heavy metals are one of the main anthropogenic factors which pollute soils (Hyroyuki,1992; Hemida, 1997; Moriarty, 1999). Their negative action on microbial cenosis and soil, biological activity is wellknown (Andreyuk, Valagurova, 1992). In this connection, the organization and carrying out of regularity control of soil state for the purpose of critical situation detecting and prognosing are actual.

Earlier we experessed an idea about a hierarchical system of the biological indication of soils polluted by heavy metals (Andreyuk te al., 1997) It consists in registration of response to pollution of soil biota at various levels of

its organisation: precell, cell, population and cenotic. It is necesary to reveal the most sensible to heavy metals index for all of these levels.

Our article is dedicated to investigations connected with elaboration of conception of microbiological monitiring of heavy metals polluted soils.

Materials and Methods

Soil Experiments were carried out at grey podzolic soil. Soil pollution by heavy metal were artificial modeled.

Heavy metals. Ion metals in doses from 1 to 20 Maximum Permissible Concentrations (MPC) were used as the solutions of salts. Accordingly to 1 MPC these salts were the next ($mg \cdot kg^{-1}$ soil) : $CdSO_4$ - 5.56, $CuSO_4 \cdot 5H_2O$ - 11.8, $Pb\ Cl_2$ 26.8, $ZnSO_4 \cdot 7\ H_2O$ - 101.1, $Hg(NO_3)_2 \cdot 2\ H_2O$ - 2.8, $SrCl_2 \cdot 6\ H_2O$ - 243.3. Control is a soil without metals.

Meliorants There were esed the next meliorants: white and green zeolite (by 3 t/ha), vermiculite (0.5 t/ha), biohumus (8 t/ha), hyps (2.1t/ha) with *Streptomyces alboviridis 141 (*$3 \cdot 10^{10}$ CFU/ha), KC-1 (Enposan) on the basis of microbial polysaccharide ($15 \cdot 10^3$ l/ha)/

Soil biological activity. Ascorbatoxidising activity of soil was determined by modified method (Iutynska, Seraya, 1996); soil mutagenic activity - by calculation of *Salmonella typhimurium TA100* frequency mutatuins (Ames, 1979) nitrogenfixing activity - by acetylene-reductase method (Hardy et al., 1968).

Microorganisms. He number of microbes of various ecological-trophical and taxonomical groups were counted according to to general acceptad methods of soil microbiology (Methods*..., 1991; Kalininskaya, 1981).

He data were statistically processed by general accepted methods (Rokitsky, 1961).

Results and Discussion

The activity of respirator enzyme ascorbatoxidase in soils is determined at the precell level. From Fig.1. one can see the average value of soil ascorbatoxidszing activity under experimental heavy metal pollution in dynamic.

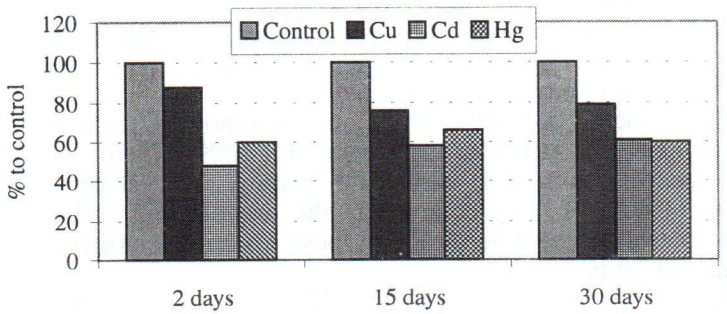

Fig.1. Ascorbatoxidizing activity of soil polluted by hevy metals

It is shown that pollutionof gray podzolic soil by Cu^{2+} and Cd^{2+} in a doze of 15 $mg \cdot kg^{-1}$ of soil significantly inhibits enzyme activity by 18-45% in comparison to control on the whole.

Determination of ascorbic acid oxidation rate in sterile and nonsterile soil permit to reveal not only the presence of heavy metal ions in polluted soil but the degree of their toxic effects on soil enzymatic activity. Perspectives of use the ascorbatoxidizing activity as one of diagnostic indices characterizing soil pollution by heavy metals is evident.

The indicator characteristic at the cell level, in our opinion, is the effect of pollutants on genom of microorganisms. In pther words, heavy metals as a mutagenic factor.

The mutagenous effect of soil polluted by heavy metals has been studied. It was shown thah the polluted soil has a definite mutagenic action on the cells of *Salmonella typhimurium TA10* auxotroph mutant (Fig.2) Pb^{2+} as separate metal revealed the highest level of mutagenic action: the mutation frequency of *S. typhimurium TA 100* exceeded the spontaneous level of mutation in 2.1 times. The mutagenic action of Cu^{2+} , Zn^{2+} or Cd^{2+} was not statistically autentic. Mixture of metals revealed the most mutagenic action than any separate metal Test-culture mutation frequency under pollution by mixture of metals increased in 7.8 times in comparison to control.

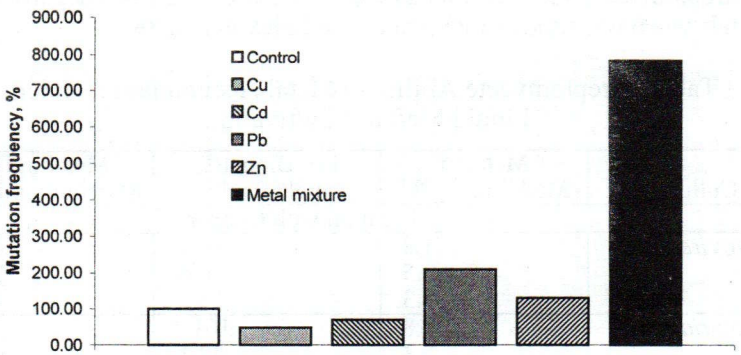

Fig.2. Heavy metals mutagenic action on *S. typhimurium* TA100

Use of meliorants significantly decreased the mutagenic action of polluted soil on the whole. White and green zeolites, biohumus, vermiculite or hypsum with *S.alboviridis 141* decreased this action to spontaneous level of mutation under low doses of pollution (1MPC). Under the high level of pollution the effective protection revealed only biohumus and hypsum with streptomycete.

The investigation at the population level were connected with the study of certain species of soil microorganisms (streptomycetes, legume bacteria, azotobacter).

Streptomycetes is one of the main components of soil microbial cenosis. They are widespread in nature and well known as producers of biologically active substances - antibiotics, enzymes, lectins etc. It is known that streptomycetes are among microbes which sensitive to the action of heavy metals. At

the same time the heavy metal effect on the streptomycetes is studied insufficiently. According our data the metal effect was most clear on the second day after their entrance in soil and depended not only on the metal itself, but also on its dose. The reconstruction of qualitative structure in the streptomycetes association was observed under the metal action. Quantity of *Achromogenes* streptomycetes was increased. Under the action of Cu^{2+}, Sr^{2+} or Pb^{2+} the quantity of *Voilaceus* and *Fuscus* streptomycetes considerably decreased; separate representatives of *Aureus, Flavus, Helvolus,* and *Albus,* which were absent in the non-polluted soil were appeared. On the whole, cadmium and mercury proved to be most toxic in respect to streptomycetes under the investigated doses of metals.

Our experiments revealed sensitive and resistant cultures of streptomycetes to heavy metals. *S.versipelis 1, S.griseoalbus 6, S.viridogenes 570* are the members of first group. The increasing of metal content in the solid medium caused to decrease of their colony quantity; the colonies became shallow and lost the aerial mycelium, capacity for sporeforming and soluble pigment, and, at last, died. These cultures may be use as indicators of the soil heavy metal pollution. The resistant forms had good growth under experimental conditions and able to accumulate the metals to 653mkg·g^{-1} dry mycelium (tab1). It is possible, that these streptomycetes are able temporarily remove the metals out the circulation and decrease the negative influence to plants. It is not surprised that one of discovered resistant streptomyces, such as *S.alboviridis 141*, decreased the negative effect of heavy metals when was used as meliorant in complex with hyps.

Tab.1. Streptomycete Ability to Metal Accumulation under Liquid Medium Cultivation

Culture	Metal in Medium, mg?l^{-1}	Lry Biomass, mg?50 ml^{-1}	Metal in Dry Micelium, mkg?g^{-1}
Medium with Copper			
S.flavovirens 12	1.8	84.9	151.9
	3.5	76.6	229.8
	5.3	52.3	184.7
S.alboviridis 141	1.8	101.4	93.7
	5.3	88.1	123.7
	7.1	84.8	213.4
Medium with Cadmium			
S.albaduncus 279	1.7	257.7	107.5
	3.4	136.6	154.4
	5.1	128.9	203.2
S.alboviridis 141	1.7	93.9	150.2
	3.4	92.7	131.6
	5.1	75.6	132.3
Medium with Plumbum			
S. sp. 407	2.6	104.4	55.5
	5.3	140.6	74.7
	7.9	97.6	124.0
S.alboviridis 141	2.6	134.0	137.3
	5.3	125.0	625.8
	7.9	125.0	463.2

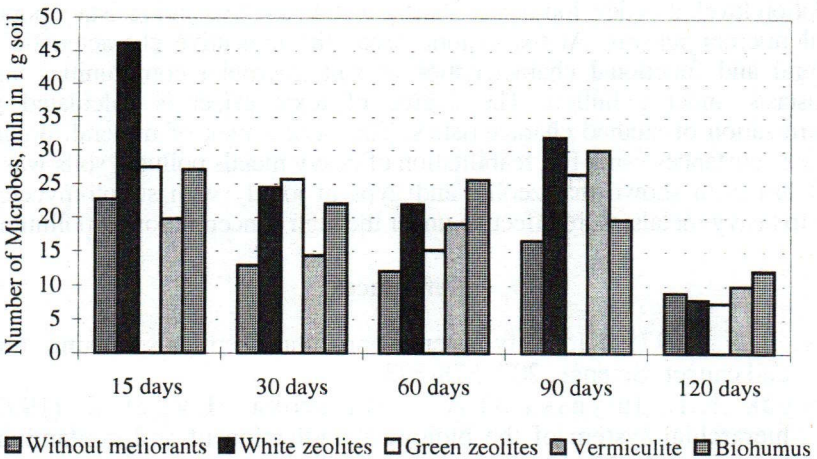

Fig.3. Influence of Meliorants on Development of Microbes Resistant to Heavy Metals.

Conclusion

The hierarchic system of microbial characteristics for monitoring of heavy metals polluted soils has been elaborated (tab.4) and tested.

Tab.4. Index System for Microbiological Monitoring of Soils Polluted by Heavy Metals

Hierarchical Level	Indicator Index	Biotest
Precell	Soil Enzyme Activity	Ascorbatoxidase
Cell	Toxicity and Mutegenic Activity of Polluted Soil	*Salmonella Typhimurium TA 100*
Population	Phisiological Reaction of Sensitive Culture (Growth)	*Azotobacter Chroococcum; Streptomyces versipelis, S.griseoalbus, S.viridogenes*
Cenosis	Homeostasis of Soil Microbe Communities	Survivability of Microbes at Polluted Soil; Sensitivity to Pollutants; Period of Microbial Number Restoration
	Activity of Legume Rhizobial Simbiotic System	Nodulae Activity of *Rhizobium* at Polluted Soil

It takes into consida(e)ration the response of soil biota at various levels of its organization. At theprecell level, the activity of reapirator enzyme ascorbatoxidase in soils is determined. At the cell level, the indicative characteristic is the pollutant

mutagenous effect on the test-organism cells- *S.typhimurium TA 100.* At the population level, it is development of heavy metal sensitive and resistant populations of soil microorganisms. At the cenotic level, the indicative characteristic are the structural and functional characteristics of soil microbial communities and their himeostasis under pollution. The degree of toxic effect is calculated via the stendardization of studied characteristics. The assessment of mineral, organic and complex substances using for reabilitation of heavy metals polluted soils was carried out. It has been shown that zeolite and gyps in jointly with streptomycete strain, stable to heavy metals, were effective under the high concentration of pollution.

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MIKROBIOLOŠKI MONITORING ZEMLJIŠTA ZAGAĐENIH TEŠKIM METALIMA I TEHNOLOGIJA NJIHOVE SANACIJE

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Rezime

U ovom radu je ispitivan hijerarhijski sistem mikrobioloških karakteristika zemljišta zagađenih teškim metalima. On je zasnovan na mikroorganizmima osetljivim na teške metale kao i njihovoj fiziološkoj i biohemijskoj aktivnosti pri različitim stepenima njihove organizovanosti: predćelija, ćelija, populacija i cenoza. Ispitivane su i neke mere popravke zemljišta u cilju rehabilitacije zemljišnog zagađenja.