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INFLUENCE OF HECHACHLOROBENZENE ON MICROBIOTA OF CHERNOZEM SOIL

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Many research has been devoted to the influence of organochlorine pesticides (OCP) on soil microorganisms, but there is little information available on effect of different levels of contamination with hexachlorobenzene (HCB) on soil microbiota. ***The purpose of this study*** *was to investigate the influence of various doses of HCB on microbiota of chernozem soil.* ***Methods****. Quantity of microorganisms was assessed using the method of sowing tenfold dilutions of the soil suspension on agarified nutrient media and assessing the number of colony-forming units (CFUs). The* ***results*** *of the research showed that the HCB effect on microbial quantity of functional groups and their ratio was negative. The most susceptible to HCB action were cellulolytic fungi and streptomycetes, the most stable ones − soil micromycetes.* ***Conclusions.*** *HCB effect disrupts both the mechanisms as resistance and flexible stability of microbial communities.*

Keywords: hechachlorobenzene, soil microniota, resistance, flexible stability, pedotrophics, amylolytics, oligonitrotrophic and nitrogen-fixing bacteria, phosphate-mobilizing bacteria, streptomycetes, cellulolytic fungi, micromycetes.

Microorganisms play an important role in functioning of agroecosystems. There are many functions that microorganisms performing, such as participating in the nutrient cycles, and increasing of soil fertility. They may also affect the spread of phytopathogens and plant resistance to them. Prokaryotes are a very important component of soil biota, due to their quantity, biodiversity and high functional activity. Intensive system of plant growing that remains a dominant factor in agricultural production causes change in the abundance and biodiversity of soil microbiota due to using chemical means for plant protection. Unlike other xenobiotics, organochlorine pesticides (OCP) are unique chemical stressors. These substances can be stored in the soil for a long time and affect the state of the ecosystem. Due to high chemical toxicity and solubility in lipids OCP accumulate in the fatty tissue of animals and thus proceed to higher trophic levels [1]. In addition, they can be transferred over a long distance by circulating in the atmosphere from low to high latitudes. Many OCP, including hexachlorobenzene (HCB) are known to have been found in soils and animals from the most remote Arctic and Antarctic regions [2]. One of the most common organochlorine pesticides is HCB, which is component of industrial waste. Currently HCB is prohibited in most countries for its negative impact on human health and the environment [3]. Despite the prohibition of HCB production in the USA and Europe, it is an important by-product or intermediate in the manufacturing of some pesticides [4]. The significant amount of current environmental pressure of HCB is also believed to be concentrated in the soil [5]. The predominant amount of HCB in the atmosphere is believed to evaporate mainly from contaminated soils [2].

Soil microorganisms are most sensitive to pesticide contamination. As part of a soil microbiota, there are populations that are suppressed or eliminated, as well as more tolerant, becoming dominant, having their high ecological plasticity. Microbial strains resistant to pesticide contamination may be promising in soil bioremediation [3]. Stability of soil microbiota from various agrocenoses in Ukraine to HCB contamination is insufficiently investigated.

Therefore the primary objective of this work was to study the influence of hexachlorobenzene as component of industrial waste on microbiota of chernozem soil from Kyiv region.

Materials and methods. Experiments were conducted in laboratory conditions. Soil weights of 250 g were placed in plastic containers, moistened to 70% of the total moisture content, maintained for 12 days to stabilize biological processes, and then experimented with soil contamination by hexachlorobenzene (HCB) in such maximum permition concentration (MPC): 10, 500, 1000, 5000, 10000, calculated according to MPC of hexachlorobenzene is 0.08 mg/kg of soil [6]. Researched industrial waste contains 90% HCB and 10% various industrial impurities [7]. Control variant was without HCB toxicant. Experiments was performed in two replications. Soil incubation was carried out for 60 days at +18 +20°C, maintaining 70% of the total moisture content. On the 45th and 60th day of the experiment microbiological studies of the soil were carried out. The quantity of microorganisms was assessed by the method of sowing ten-fold dilutions of the soil suspension on agarified nutrient media and assessing the number of colony-forming units (CFUs) per gram of dry soil, taking into account its moisture content. The quantity of pedotrophic microorganisms was determined on soil agar, amylolytic – on starch-ammonia, oligonitrotrophic and nitrogen fixing – on the Ashby's medium, phosphate-mobilizing − on the Menkina's medium with phenolphthalein sodium phosphate, streptomycetes − on potato-glucose agar (PGA), cellulolitic – on the Hutchinson's medium with a disk of filter paper, micromycetes – on the Czapek's medium[8]. Statistical processing of the results was carried out using Excel software.

Results. As a result of the study of the effect of various doses of HCB on microbiocenosis of chernozem soil, data have been obtained that HCB has a pronounced toxic effect on all functional and taxonomic groups of microorganisms.

Pedotrophic bacteria under the action of low dose HCB (10 MPCs) decreased their quantity by 11.5% after 45 days of cultivation, and after 60 days − by 34.5% compared to non-contaminated control (Fig.1 A). At the action of the average dose of HCB (500 and 1000 MPCs), their number decreased by 31.8% and 59.5%, according to 45 day and 60 day cultivation. In the variant with an ultrahigh dose of a toxicant 10,000 MPCs after 45 days the content of pedotrophic bacteria decreased by 55,4%, and after 60 days − by 67,3%. Amylolytic bacteria have shown a relative tolerance to the HCB contamination. Under the action of polluting doses in the interval from 10 to 500 MPCs, their number decreased by 25%. However, in conditions of increasing the dose up to 1000 MPCs and subsequent toxic load up to 10,000 MPCs, their number fluctuated from 47 to 53% compared to non-contaminated control (Fig.1 B).

A

B

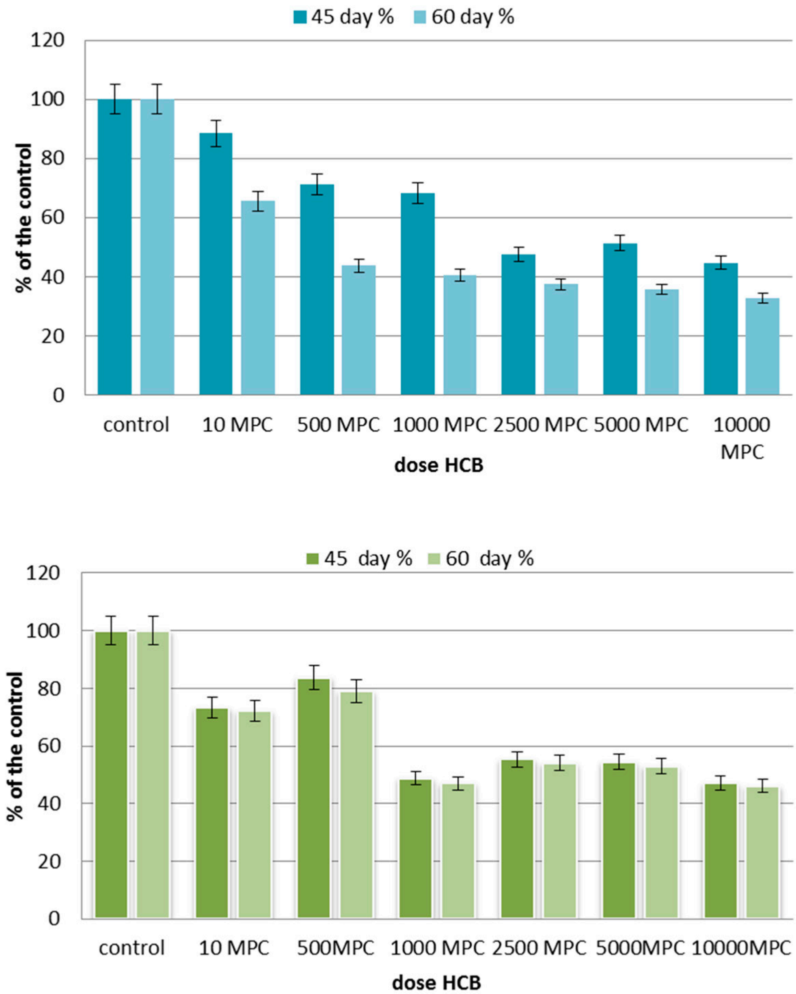

Fig. 1. Quantity of pedotrophic (A) and amylolytic (B) bacteria in the soil contaminated with different doses of HCB

Oligonitrotrophic and nitrogen-fixing bacteria in the first 45 days of the experiment showed resistance to HCB in the dose range from 10 to 1000 MPCs, for which the quantity slightly decreased by 14.2 – 17.2% as compared to the control. However, in these variants during 60 days, the number of oligonitrotrophic and nitrogen-fixing bacteria decreased by 42.2 – 43.3%. In the case of high contaminating doses (2500 − 10000 MPCs), the number decreased in the first 45 days by 48.8 – 55.7% in comparison with control and has remained at this level for the next 60 days of cultivation (Fig. 2A).

Phosphate-mobilizing bacteria demonstrated relative tolerance to HCB. At the smallest dose of contamination, their quantity decreased by only 14.6% compared to non-contaminated control, and at the highest dose of contamination, their number remained high enough: 52.1 – 53.2% compared to control. It should be noted, that the quantity of phosphate-mobilizing bacteria in the course of cultivation (from 45 to 60 days) was statistically unreliable. It can be suggested that phosphate-mobilizing bacteria under long-term action of HCB were adapted to its toxic effects (Fig. 2B).

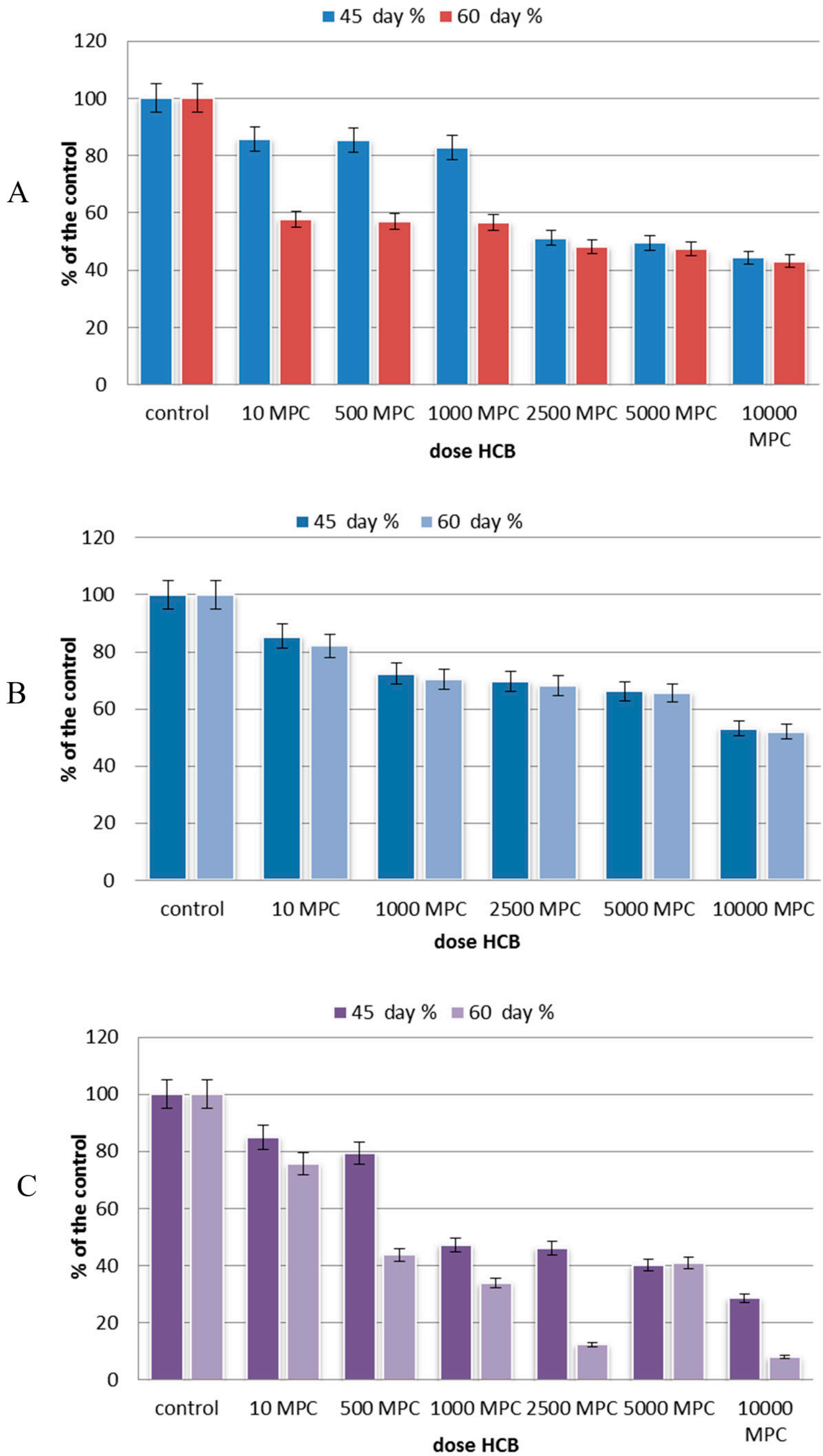

Fig. 2. The number of oligonitrotrophic and nitrogen-fixing (A), phosphate-mobilizing (B) bacteria and streptomycetes (C) in soil contaminated with different doses of HCB

On the 45th day of the experiment streptomycetes showed a direct dependence of the number on the toxicant amount. Compared with control level over 45 days of the experiment at low dose of 10 MPCs contamination, the quantity of streptomycetes decreased by 15.1%, under the action of 1000 MPCs – by 53%, and 10,000 MPC − by 71.4%. In conditions of longer-term exposure to HCB (60 days), a further significant decrease in the number of streptomyces was detected. Even with the smallest dose (10 MPCs), its number decreased by 24.4%, under the action of 1,000 MPCs − by 66%, and 10000 MPCs − by more than 92% (Fig. 2B).

Cellulolytic fungi also appeared to be sensitive to the effects of various doses of HCB. Their number decreased in direct proportion to the dose of HCB for both 45 days and 60 days of experiment. Under the influence of 10 MPCs its number decreased by 46.9 − 48.2%, 1000 MPCs − by 76 – 76.4%, 10,000 MPCs − by 87.9 − 88.2% compared to the control (Figure 3A).

Soil micromycetes appeared to be the most tolerant to HCB, as even under the action of the highest dose HCB (10,000 MPCs) their quantity decreased by only 24.4% compared to the control. After 60 days of cultivation in the presence of different doses of HCB in comparison with 45 days, no statistically significant diminution in the number of micromycetes occurred (Fig. 3B).

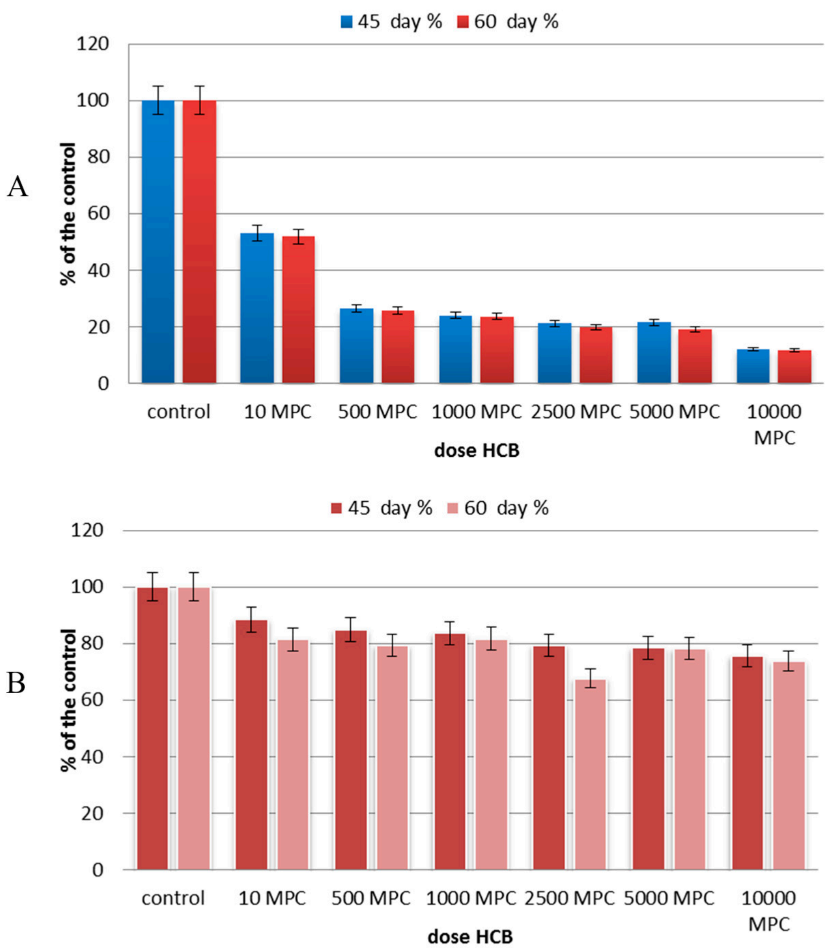

Fig. 3. The number of cellulolytic fungi (A) and micromycetes (B) in soil contaminated with different doses of HCB

Generalized data on the reaction of microbial communities on HCB effect are presented in Fig.4.

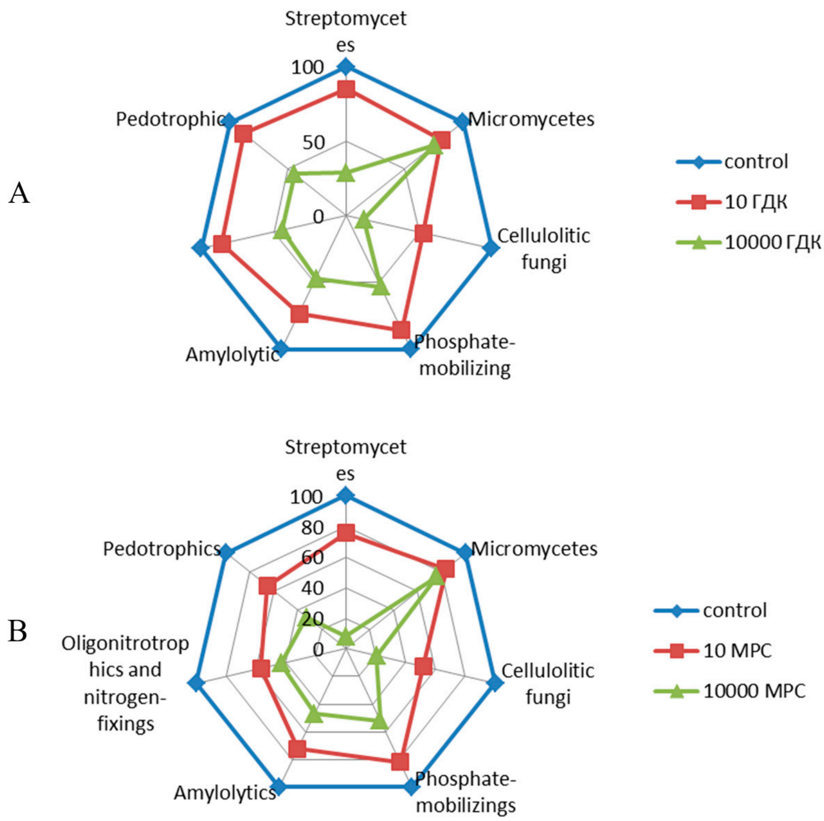

Fig. 4. Effect of HCB on soil microorganisms of different functional groups (% to the control): A – cultivation for 45 days, B – cultivation for 60 days

In the course of cultivation for 45 days under the action of the smallest dose 10 MPCs of pollution with a slight decrease in the number (12 – 17% compared to non-contaminated control) showed relative stability in the ratio of the number of functional and taxonomic groups of microorganisms with the exception of a significant decrease (by 47%) of the amount of cellulolytic fungi. Under the action of a super-high dose of contamination (10000 MPCs), the structure of the microbial communities compared with non-contaminated control varied toward a significant reduction in the number of streptomycetes (by 71,4%) and cellulolytic fungi (at 87,9%).

With an increase in the duration of HCB influence to up to 60 days in the variant with a dose of 10 MPCs, there was a further reduction in the number of microorganisms compared with 45 days cultivation, but their ratio did not change significantly compared with the control, except for decrease in the number of cellulolytic fungi.

The highest dose of contamination of 10,000 MPCs provoked changes in the ratio of microorganisms quantity, which consisted in a significant reduction in the number of streptomycetes and cellulolytic fungi (up to 92% and 87.9% respectively), and the preservation of high levels of soil micromycetes.

Discussion. The obtained results revealed that pedotrophic bacteria are sensitive to the action of HCB, and long-term effects of high doses of toxicants significantly reduce their number.

In contrast to pedotrophic bacteria, oligonitrotrophic and nitrogen-fixing microorganisms established tolerance in a wide range of toxic loading, i. e. with an essential increasing of contaminating doses, no significant reduction in the number was detected compared with the lowest contamination doses.

It is known that the majority of streptomycetes are the sensitive group of soil microorganisms to organochlorine pesticides, especially to its long-term pressure effect [9]. The decreasing number of streptomycetes during exposure to HCB indicates that the sustainable development of chernozem soil streptomycetes under the action of HCB is violated. Such type changes can negatively affect the functions of soil microbiota in general. Every soil microbial group provides essential transformative and biosethetical processes [10]. Streptomycetes are known to be producers of a wide range of biologically active substances (antibiotics, phytohormones, lipids, vitamins), which have a positive effect on plants and their resistance to diseases [11].

Cellulolytic fungi play an important role in the transformation of plant residues and humus formation [12].

The response of soil micromycetes to pesticidal loading was radically different. The prevalence of micromycetes against reducing the quantity of prokaryotes of different functional groups can lead to deterioration of the soil phytosanitary state since among the micromycetes there are many phytopathogenic representatives.

It should also be noted that in our experiments, in any case, we did not observe the tendency to recovery of the microorganisms quantity. It is known that in the ecology "stability" is defined as a dynamic response to stress, that is, the ability of a system to self-regulation, which manifests itself in two forms: flexible, such as the rate of return of a system to the initial state and resistance, as the ability of a system to avoid changes [13, 14].

Proceeding from these provisions, as well as from our experimental data, we can conclude that the contamination of chernozem soils by HCB at doses from 10 to 10,000 MPCs violates the mechanisms of resistance and flexible stability of microbial communities.

The most susceptible to HCB contamination are cellulolytic fungi and streptomycetes, the most stable − soils micromycetes. The microbiocenosis structure and the number ratio of major ecological trophic groups, mechanisms of resistance and elastic stability of microbiota are violated in HCB contaminated soil.

ВПЛИВ ГЕКСАХЛОРБЕНЗОЛУ НА МІКРОБІОТУ ЧОРНОЗЕМНОГО ҐРУНТУ

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Резюме

Питанню про вплив хлорорганічних пестицидів на ґрунтові мікроорганізми присвячено багато робіт, але дію різних рівнів забруднення гексахлорбензолом на мікробні угруповання ґрунту ще недостатньо досліджено. **Метою** роботи було вивчення впливу різних доз ГХБ на мікробіоту чорноземного ґрунту. **Методи**. Кількість мікроорганізмів визначали методом посіву ґрунтової суспензії на агаризовані поживні середовища з наступним підрахунком кількості колоній утворюючих одиниць (КУО). **Результати** дослідження показали, що ГХБ негативно вливає на чисельність еколого-трофічних груп мікоорганізмів та їх співвідношення у мікробіоценозі ґрунту. Найбільш чутливими до забруднення були целюлозолітичні гриби і стрептоміцети, найбільш стійкими – ґрунтові мікроміцети. **Висновки**. За дії ГХБ порушуються механізми стійкості опору і пружної стійкості мікробних угруповань.

Ключові слова: гексахлорбензол, мікробіота ґрунту, стійкість, гнучка стабільність, олігонітротрофні та азотфіксуючі бактерії, фосфатмобілізуючі бактерії, стрептоміцети, целюлозолітичні гриби, мікроміцети.

ВЛИЯНИЕ ГЕКСАХЛОРБЕНЗОЛА НА МИКРОБИОТУ ЧЕРНОЗЕМНОЙ ПОЧВЫ

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Резюме

Вопросу влияния хлорорганических пестицидов на почвенные микроорганизмы посвящено много работ, но действие различных уровней загрязнения гексахлорбензолом на микробные сообщества почвы еще недостаточно исследовано. **Целью работы** было изучение влияния различных доз гексахлорциклобензола (ГХБ) на микробиоту черноземной почвы. **Методы**. Количество микроорганизмов определяли методом посева почвенной суспензии на агаризованные питательные среды с последующим подсчетом количества колоний образующих единиц (КОЕ). **Результаты** исследования показали, что ГХБ негативно влияет на численность эколого-трофических групп микроорганизмив и их соотношение в микробиоценозе почвы. Наиболее чувствительными к загрязнению были целлюлозолитические грибы и стрептомицеты, наиболее устойчивыми – почвенные микромицеты. **Выводы**. Под действием ГХБ нарушаются механизмы резистентной и упругой устойчивости микробных сообществ.

Ключевые слова: гексахлорбензол, микробиота почвы, устойчивость, гибкая стабильность, олигонитротрофные и азотфиксирующие бактерии, фосфатмобилизирующие бактерии, стрептомицеты, целюлозолитические грибы, микромицеты.

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