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CHAPTER

Screening of fungal strains resistant to heavy metals 11

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Abstract

The monograph discusses the research concerning the screening of strains of fungi resistant to heavy metals. The entry of heavy metals into the soil in large quantities affects the biological and biochemical properties of soils, and changes the amount of mobile forms of nutrients in them. Soil pollution with heavy metals affects the transformation of nitrogen-containing substances, inhibits the activity of nitrogen fixation, etc. The decrease in the qualitative and quantitative composition of agricultural products due to the impact of heavy metals requires the introduction of new, highly efficient agricultural practices. In many ways, the deterioration of agricultural land and the decline in the quality of agricultural products are associated with the spread of infectious plant diseases. Currently, research is being conducted in biotechnology aimed at using microorganisms as tools for removing or concentrating heavy metals from polluted environmental objects. This technology is based on the ability of cells of certain microorganisms to accumulate heavy metals in large quantities from soil and water environments. Among the many toxicants entering the natural environment, heavy metals are of particular importance. In one quantity or another, they have always been contained in soils and natural waters. Entering the biosphere, heavy metals are actively involved in migration cycles and accumulate in various components of ecosystems, including hydrobionts. The particular danger of accumulation of heavy metals is that, unlike toxicants that are of an organic nature and decompose to a greater or lesser extent in natural waters, heavy metal ions are constantly stored under any conditions.

Keywords: Heavy metals; MIC; minimum inhibitory concentration; MTC; maximum tolerant concentration

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s0010 Abbreviations

HM heavy metals
MIC minimum inhibitory concentration
MTC maximum tolerant concentration

s0015 11.1 Introduction

p0030 At present, the ecological safety of the environment is one of the priorities of the national policy of several countries. One of the sources of contamination of organic ecosystems are by-products of machine-building, metallurgical, automotive, instrument-making, and other industries, containing a significant amount of contaminants, which include various organic agents, alcohols, acids, surfactants, and oil products, as well as high concentrations of heavy metal ions. The composition of such contaminants is extremely diverse, it changes in the process of the emergence of new industries and the improvement of existing ones. In recent decades, environmental pollution by heavy metals has been increasing globally. Because of this, the increase in their content in the soil, atmosphere, and water becomes a serious environmental problem. Defilement of farm land and groundwater with heavy metals is directly related to human activities. The danger of an increase in the content of heavy metals in the soil and atmosphere is also associated with their active absorption and accumulation in plants, which not only negatively affects the vital activity of the plants themselves, but also poses a serious menace to human and animal health (Behrooz and Gholamalizadeh, 2014).

p0035 High concentration of heavy metal ions in the environment leads to their absorption by plant roots and entry into the aboveground plant parts, causing metabolic disorders and stunted growth (Munees, 2014). High content of heavy metals in polluted soils leads to decrease in fertility and, ultimately, to a decline in prolificness.

p0040 Therefore, at present, the problem of resistance and adaptation of plants to properties of heavy metals is given great attention globally. In this regard, the study and use of microorganisms with different types of biological activity, resistance to heavy metals, and the ability to concentrate and extract them from the environment is of great scientific and practical interest.

p0045 Currently, the problem of soil contamination with metals is quite acute. Plants and microorganisms have the ability to accumulate and remove metals. Of greatest interest are microorganisms, in particular fungi, which are well known for the accumulation of metals and have a number of useful properties, which makes it possible to use them for the neutralization and accumulation of metals. The effects of metals on the macro- and micromorphology of filamentous fungi and yeasts have not been fully studied.

p0050 In modern conditions, the objective of agricultural production is the biologization of agriculture, which is based on agricultural practices that provide environmentally friendly food, reduce environmental pollution, and preserve soil fertility. The conducted studies are of practical value for the elaboration of economically profitable agricultural production, as well as for finding ways to solve a number of ecological problems. The outcomes of the research can contribute to the effective use of the potential of microorganisms to magnification of the resistance of plants to stressful factors, which can become the base for the elaboration of technology for growing plants under the impact of heavy metals.

s0020 11.1.1 Environmental features of heavy metals

p0055 The storage of high concentrations of heavy metals in the environment can cause a lot of damage to human health and serious environmental problems. Currently, bioremediation using microorganisms is gaining a lot of consideration due to its satisfactory performance. The soil is a compound ecosystem wherein various microorganisms play an important role in maintaining soil fecundity and plant performance through interaction with both biological and physicochemical components (Behrooz and Gholamalizadeh, 2014; Munees, 2014; Gergely et al., 2016; Roman-Ponce et al., 2017).

p0060 Heavy metal contamination is one of the major environmental issues in nautical, terraneous, and freshwater areas. It can accumulate in the human body through the food chain, thus contributing to chronic and acute disorders and apparently serious health issues. Anthropogenic springs of metal pollution can be separated into five main groups:

o0010 **1.** Metallurgical extraction and smelting
o0015 **2.** Industries
o0020 **3.** Atmospheric indication
o0025 **4.** Agriculture
o0030 **5.** Waste removal

p0090 Industries bearing heavy metals, such as Pb, Zn, Cr, Cd, Cu, As, and Ni, are a significant problem as they are known to have long-term negative impacts on environmental soil and water. The resistance mechanisms include the exclusion of metal species from the surface of microbial cells, the bioaccumulation of metal ions within the cell energetically or passively, the biotransformation of toxic metals to less toxic forms, and the adsorption of metals on the cell wall. Therefore, it has been shown that bacterial strains isolated from contaminated environments can tolerate higher metal concentrations than those isolated from uncontaminated areas. Microorganisms have developed a whole complex universal and specific resistance mechanisms with respect to a certain stressor (Lyudmila et al., 2021).

p0095 . The biosynthesis of regulatory proteins of various functionalities, such as chaperones and s-factors, is one of the universal responses to stress. Chaperones are involved in maintaining the integrity and stability of DNA and RNA, transcription and translation, DNA repair, protein structuring, and the normal functioning of enzymes. s-Factors regulate the stabilization of cell membranes, the biosynthesis of exopolysaccharides, the transition of the cell to the stationary phase, etc. Nonspecific resistance is also provided by the biosynthesis of low molecular weight osmoprotectants (glycine, glycine betaine, proline, trehalose, glutamate, taurine, acetylglutaminyl, glutaminamide), exopolysaccharides, and polyamines (Odokuma1 and Akpona, 2010).

p0100 It has been established that carboxyl groups on the surface of bacteria of the genus *Pseudomonas* ensure the binding of up to 100 mcg of Cd per 1 mg of biomass. Heavy metal immobilization can also take place outside microbial through changes in the pH and redox potential of the medium, mobilization of phosphates, or manufacture of polysaccharides, siderophores, and other substances.

p0105 Thus, heavy metals disrupt the complex of processes in the plant and induce many specific and nonspecific reactions (Aryal, 2020). At the same time, microorganisms have beneficial properties for plants, a favorable combination of which can have an additive or synergistic effect in a stressful situation. This must be taken into account in fundamental research when explaining the results of experiments and in the practice of using bacteria for plant inoculation. On the one hand, the multifunctionality of individual strains and the dependence of bacterial properties on environmental factors, which often cannot be controlled. It complicates the interpretation of the results, on the other hand, it opens up opportunities for combining many useful functions in one strain during selection for several traits or genetic transformation of metals. Studies are underway on the gears of this process, the isolation of active strains of bacteria, fungi, and algae, microbial communities are selected, and the effect of outer conditions on the bioaccumulation operation is determined. The decision of a number of environmental problems hinges not only on the ability of a number of microorganisms to precipitate or accumulate heavy metals, but also on the study of the toxic effect of the latter on microorganisms (Backer et al., 2018). It is crucial to assess the contribution of microorganisms in the transformation of heavy metal connection and detoxification of the natural environment. Research is underway on the role of metals as trace elements necessary for the life of microorganisms, as energy sources, or electron acceptors, as elements that affect the natural habitat of microorganisms.

s0025 11.1.2 Sources of soil contamination with heavy metals

p0110 Heavy metal entry into the environment is connected with human activity. Their primary sources are industry, transportation, steamshop, waste incineration plants, and farm production (Basu et al., 2021).

p0115 The industries that pollute the environment with heavy metals engage in ferrous and non-ferrous metallurgy, the extraction of solid and liquid fuels, production and processing complexes, glass, ceramic, electrical production, etc.

p0120 Lead is extensively used in the production of batteries, electrical cable sheaths, medical facilities, crystal glass, optical glass, paints, numerous alloys, etc., not to mention the production related with its production.

p0125 In rural production, soil pollution with heavy metals is linked with the use of fertilizers and pesticides (Shadmani et al., 2020).

p0130 Transportation is the source of more than half of all emissions into the atmosphere. Boilers operating on solid and liquid fuels pollute the environment not only with heavy metals, but also with various oxides. Waste burning is accompanied by the entry into the biosphere of a number of heavy metals, such as cadmium, mercury, lead, chromium, etc.

p0135 For large cities with a diversified industry, the presence in the environment is not a single pollutant, but an association of heavy metals that can have a combined effect on the body, in which both the summation of effects and their potentiation can be observed. Hazardous levels of environmental pollution with heavy metals are observed in many industrially developed areas (Shadmani et al., 2021).

p0140 The compounds of heavy metals that have entered the environment contaminate the atmospheric air, water, soil, and enter the plants, organisms, and animals inhabiting the area.

p0145 Under the influence of such an anthropogenic factor as heavy metals, there are substantial changes in the morphological, anatomical, biochemical, and physiological parameters of microbial cells. The nature of the modifications is to a large degree determined by the properties of heavy metals, their concentration, and outer conditions (pH, aeration, salt regime). Simultaneously, the peculiarity of responses to the action of toxicants relies on the systematic position of microorganisms, the age and physiological position of the culture, the activity of enzymes, and the characteristics of exometabolites (Xu et al., 2021).

p0150 Specialists in environmental defense have matched a priority group among toxic metals. The priority group includes cadmium, copper, arsenic, nickel, mercury, lead, zinc, and chromium, which are the most dangerous for human and animal health. Of these, mercury, lead, and cadmium are the most toxic.

p0155 Soils can be contaminated with heavy metals through emissions from rapidly developing industrial areas, tailings, disposal of heavy metal waste, leaded gasoline and paints, fertilization, manure, sewage sedimentation, pesticide residues from coal combustion, oil spills, and atmospheric deposition (Tomei et al., 2021). Heavy metals are a fuzzy group of inorganic chemical hazards, and the most commonly encountered in contaminated sites are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni). Soil is the main sink for heavy metals released into the environment by the aforementioned anthropogenic activities. Unlike organic pollutants, which are oxidized to carbon (IV) oxide by microbial action, most metals do not undergo microbiological or chemical degradation. The total concentration in soils remains for a long time after their introduction. However, changes in their chemical forms (speciation) and bioavailability are possible. The presence of toxic metals in soil can seriously inhibit the biodegradation of organic pollutants. Soil contamination with heavy metals can pose a risk to humans and the ecosystem through direct ingestion or contact with contaminated soil, food chain (soil-plant-human or soil-plant-animal-human), consumption of contaminated groundwater, and reduced food quality (safety and competitiveness). Due to phytotoxicity, land use for agricultural production is reduced, which causes food shortages and land use problems. The use of chemicals in agriculture entails a negative anthropogenic load on agrophytocenosis, leads to pollution of natural resources of the agroecosystem with heavy metals and other toxicants, while distorting the biological characteristic of the soil and reducing the productivity of arable land. Therefore, to obtain environmentally friendly products, it is very important to evaluate the use of chemicals for agriculture in the Almaty region, in terms of the accumulation of heavy metals in the soil. Solving the problem of environmental efffects due to the use of mineral fertilizers is possible only on the basis of a systematic approach that takes into account both the positive and negative effects of this method of increasing soil fertility (Carlos et al., 2016).

p0160 Mineral fertilizers are one of the basic sources of soil contamination with heavy metals and toxic elements. This is associated with content of zinc, lead, strontium, cadmium, lanthanides, and other chemical elements in the raw materials used for the production of mineral fertilizers. Their total elicitation is or not provided at all, or is determined by technological factors. The set of heavy metals, and their amount in fertilizers, depends on the natural raw materials used for the production of products, its composition, determined by the geological origin and geographical location of the deposit. For example, phosphate raw materials contain cadmium, lead, and a number of other elements. The content of heavy metals in different forms of phosphate fertilizers varies greatly: from minimal amounts to 100 mg/kg. At low concentrations, most heavy metals do not have a pronounced negative effect on the life of plants, animals, and humans; however, with an increase in their concentration, many of them become toxic to living organisms. Though, as a general rule, the content of heavy metals in phosphate fertilizers is relatively low, their widespread use for agricultural crops of great economic importance often leads to a significant accumulation of heavy metals in soils. Mineral fertilizers are administered in a dose of 109 kg/ha of NPK, which contains nearly 7.87 g of copper, 10.25 g of zinc, 0.21 g of cadmium, 3.36 g of lead, 4.22 g of nickel, and 4. 77 of chromium (Soto et al., 2019).

p0165 For the entire period of using phosphate fertilizers, approximately 4200 tons of cadmium, 15,632 tons of lead, 542 tons of mercury are introduced into the soil. Most of the chemical elements (62) that have entered the soil are in a weakly mobile state. At the same time, the duration of the period for the removal of heavy metals, such as cadmium, is 111 years, zinc 512 years, copper 1400 years, and lead several thousand years. This is the ecological problem of the use of mineral fertilizers.

p0170 Soil contamination with heavy and toxic metals leads to their stockpiling in plants. Hereby, the concentration of cadmium in wheat has doubled over the current time. Ibidem, when using superphosphate in a total dose of 1570 kg/ha, attached in parts over 6 years, an enlargement in the content of cadmium in wheat grain by 4.5 times was observabled. Consequently, to some authors, soil contamination with strontium outcomed in a triple magnification in its content in potato tubers. Kazakhstan with insufficient attention has not yet paid the price of the contamination of crop products with chemical elements.

p0175 The use of infected plants as food or feed is the reason of various diseases in humans and farm animals. The most hazardous heavy metals include mercury, lead, and cadmium. The entry of lead into the human body leads to remembrance impairment and a reduction in resistance to bacterial infections. The stockpiling of cadmium in food products, the toxicity of which is 10 times higher than lead, causes the disruption of red blood cells, disintegration of the kidneys and intestines, and softening of bones and tissues. Pair and triple combinations of heavy metals enhance their toxic effect (Nazli et al., 2020).

s0030 11.1.3 Heavy metals as a factor in changes in metabolism in microorganisms

p0180 Heavy metals circulating in the atmosphere as a outcome of anthropogenic activities affect all living organisms, including the microbiota, to one degree or another. Microbial responses (MR) to stress exposures can be considered in various aspects: as examples of adaptive capabilities, as a selection factor for resistant and resistant strains, as the likelihood of being used as test organisms or indicator organisms, and, finally, as the prospect of using individual microbial groups, microbial consortiums, or plant-microbial associations for bioremediation of chemically polluted environments. Getting into the environment, heavy metal ions, first of all, collide with exometabolites of living organisms, in particular, microorganisms, and components of cell walls. In this case, the active concentration of pollutant ions in the medium can vary significantly (Mukherjee et al., 2019).

p0185 Features of the chemical structure of microorganism cells and the specifics of functioning in the presence of heavy metal ions determine their ability to change the concentration of metals in the environment. In conformity with the allocation of the process of metal biosorption, the mechanisms can be confidential as intracellular interaction, interaction on the cell superficies, and extracellular interaction. In this case, the physicochemical interaction between metal ions and MR superficies plays an important role. Biosorption is mainly a passive, metabolism-independent interaction with metals. If the batteries do not support the vital activity of the MR, then both living and dead cells are effectively used to remove heavy metals (Hu and Zhao, 2007). Many symbiotrophic microorganisms (mycorrhizal fungi, nodule, and associative bacteria) are extremely persistent to heavy metals and participate in the operations of their converting in the rhizosphere and accumulation by plants. However, the concrete mechanisms of accommodation of symbioses to toxic concentrations of metals and the unification of components of plant-microbial scheme under strained situations have not been studied enough. The problem of the cooperation of heavy metals and microorganisms is being investigated in several main directions. These issues are related to the study of changes in various biochemical, physiological, genetic, and other characteristics of microorganisms during their contact with metals in pure culture conditions. When studying the stockpiling of heavy metals by microorganisms, the methods of penetration and localization of these elements in cells, the influence of the physiological parameters of the culture, and external conditions on the storage of metals by microorganisms were established. The noxious effect of metals on the microorganism is manifested in the inhibition of their metabolism, in changes in growth kinetics and morphology. Biological transformation and partial detoxification of some of them are noted as mechanisms that opposing the resistance of microorganisms to the actions of heavy metals. Finally, among the most important problem of violation and protection of the environment from pollution by heavy metals (Gadd et al., 2015) is intracellular interaction. Active metal transport across cell membranes leads to its intracellular accumulation, which relies on the bacterial metabolism. Many heavy metals are energetically engaged by particularized intake systems, as they are necessary, but other, minor metals can also be accepted due to being erroneous for a microelement. At elevated concentrations of noxious metals, MR vigorously absorb metal ions to detoxify their habitat. The insoluble metal-containing substance precipitates as metal ions combined with various anions produced during cell metabolism. For example, *Citrobacter* sp. can accumulate high levels of uranium, nickel, and zirconium by forming metal phosphate precipitates. Sometimes, the precipitation of heavy metals is not the result of the direct action of microbial chemicals on them. Thus, in the presence of gelatin, sulfate-reducing bacteria actively produce hydrogen sulfide, the latter, in turn, precipitates almost all heavy metal ions. The precipitate can be adsorbed on the surface of cells or precipitate independently of organisms (Aziz et al., 2021).

p0190 The impact of heavy metals affects, first of all, the primary producers—microalgae and cyanobacteria. They, along with heterotrophic microorganisms, can be used to detoxify the environment from metals, since they are able to accumulate them from water and bottom sediments. Salts of silver, mercury, cadmium, nickel, copper, and cobalt disrupt the barrier properties of cytoplasmic membranes (CPM) of cells, which in turn leads to a decrease in the transmembrane potential. Under the action of silver and mercury ions, the electrophysical properties of the CPM and the cytoplasm of cells change. Vanadium, as one of the widespread heavy metals, is used by green, brown, and yellow-green algae. It is also found in the nitrogenases of some soil bacteria, although it is not a necessary element for the development of most prokaryotes. The most active bioaccumulators of vanadium are bacteria of the genus *Pseudomonas*, as well as a number of cyanobacteria. Such properties of bacteria as the ability to adapt and rapidly multiply contribute to the spread of microorganisms resistant to heavy metals. Bacteria isolated in places containing industrial pollution and in deposits of the corresponding metals have the greatest resistance to metals (Li et al., 2020). Particularly promising is the use of pigment-synthesizing bacteria of the genus *Serratia* and *Pseudomonas* as indicators of environmental pollution. Most of the heavy metals are needed in microdoses for the normal functioning of living systems. However, in case of overdose, violations of vital activity are observed, since in humans and animals their compounds do not participate in normal metabolism and their gradual accumulation leads to various diseases. Thus, these metals move to the rank of pollutants of the biosphere. Many features of microorganisms and the gears of their positive effect on plants can perform an momentous role in protecting the latter from adverse environmental conditions, since the positive effects of microorganisms are oriented, among other things, against the negative effect of stressors on plants (Zhou et al., 2021). For example, the negatory effect of heavy metals is revealed itself during the impairment of nitrogen and other nutrients consumption by plants, which under definite conditions can be the main reason for the decrease in resistance and growth inhibition. Inoculation with nitrogen fixers or bacteria that improve mineral intake will increase plant stress resistance. In plants, heavy metals summon oxidative stress, while microorganisms upon inoculation are able to activate defense reactions, enhancing, for example, proline biosynthesis and the performance of antioxidant enzymes superoxide dismutase, peroxidase, and catalase. Bacteria comprising the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase also have a universal anti-stress effect, due to which the ethylene content of the signal molecule, which triggers a cascade of nonspecific stress and adaptive reactions, decreases in plants (Zhai et al., 2022).

p0195 Microorganisms have developed a whole complex of universal and specific resistance mechanisms in relation to a certain stressor. A number of reviews are devoted to the issues of bacterial resistance to heavy metal. Several mechanisms of bacterial resistance to heavy metal ions are known: extracellular barrier, active transport of metal ions from the cell, extracellular binding, intracellular binding, and reduction of metal ions. In this case, one strain can simultaneously possess several defense mechanisms (Shahzad et al., 2021).

p0200 On the whole, there are two types of heavy metal intake mechanisms: one is fast and nonspecific, driven by a chemoosmotic gradient across the cell membrane, and therefore does not require ATP. On the contrary, the second process of metal uptake is relatively slower and more substrate-specific and depends on the energy released during ATP hydrolysis. For example, copper is used in small amounts by bacterial cells in the biosynthesis of metabolic enzymes, such as cytochrome oxidase. However, bacteria in various ecosystems, including soil and water, are often demonstrated to high concentrations of this metal because there are high levels of copper in the soil ecosystem due to its widespread use in mining, industrial processes, and agricultural practices. Consequently, bacteria have developed several types of mechanisms to protect against high concentrations of copper and its biotoxicity. Lin and Olson isolated copper-resistant bacteria from a copper catalytic water distribution system. They noted that 62% of the total number of isolates had significant resistance to copper (Ali et al., 2021).

p0205 It has been established that carboxyl groups on the surface of bacteria of the genus *Pseudomonas* ensure the binding of up to 100 mcg of Cd per 1 mg of biomass. Heavy metal immobilization can also take place outside the cells of microorganisms due to changes in the pH and redox potential of the medium, mobilization of phosphates, or production of polysaccharides, siderophores, and other substances. The capability to relate metal ions by extracellular biopolymers has been depicted in bacteria of the Pseudomonadaceae and Enterobacteriaceae families. Heavy metal sorption on the cell wall materializes substantially through exopolysaccharides, and the diameter of the mucous capsules can surpass the size of the cell. The ability to adsorb on the cell wall and absorb heavy metal ions intracellularly has been depicted for many bacteria, representatives of the genera *Arthrobacter, Klebsiella, Bacillus, Pseudomonas, Rhizobium, Serratia, Rhodopseudomonas, Lactococcus*, as well as cyanobacteria, yeasts, etc. (Vojodi Mehrabani et al., 2021).

p0210 It is very likely that substances produced by microorganisms in response to the presence of toxic metals for their immobilization or involved in the mobilization of heavy metals may show biological activity towards plants. Microbial metabolites are extremely diverse in structure and function, and many of them are involved in signaling between microorganisms and plants. Such metabolites include polysaccharides, siderophores, organic acids, and other compounds that are involved in the reactions of microorganisms to the presence of heavy metals. In this regard, it seems very interesting to study the role of these substances in plant-microbial interactions and integration of components of symbiotic systems under stress conditions (Liu et al., 2022).

p0215 In the past decade, interest in the use of rhizospheric bacteria to stimulate growth and regulate heavy metal entry into plants from polluted soils has increased significantly, which is reflected in a series of review articles. For example, industrial strains of associative bacteria *Arthrobacter mysorens* 7 and *Flavobacterium* sp. L30s diminished the mobility of Cd in the soil, and inoculation of barley with them improved plant growth and prevented the entry of heavy metals into grain. The ability of *Pseudomonas* associated with ectomycorrhizal fungi to enhance the immobilization of Cd, Zn, and Pb by the microsymbiont in roots and to prevent the entry of these metals into pine shoots was also shown. Immobilization of metals can occur due to the formation of poorly soluble complexes with bacterial siderophores, polysaccharides, and other substances. However, bacterial siderophores and their Fe-containing complexes are absorbed by plants; therefore, in some cases, an increase in the supply of metals to the latter is possible. Data have been obtained on the simultaneous stimulation of growth and nickel removal by *Sarepta mustard* (Brassicajuncea) upon inoculation with the *Bacillus subtilis* SJ-101 strain, which produces auxins and dissolves phosphates. In these and many other studies, increased absorption of toxic metals occurred without negative consequences for plant growth, which indicates the ability of bacteria to increase the homeostasis of the microbial–plant system (Girma, 2015).

p0220 The study of microorganisms associated with plants—metalophytes, heavy metal accumulators, and hyperaccumulators is mainly aimed at finding methods to improving efficiency of metal phytoextraction to clean contaminated soils

(Wei et al., 2021). In this regard, the problem of improving plant growth on toxic soils and finding ways to enhance the accumulation of toxicants in the aerial parts of plants is an urgent task. Inoculation with microorganisms, whose action is aimed at stimulating plant growth, seems to be a promising approach for intensifying phytoextraction. It is also known that microorganisms are able to mobilize metals because of environmental acidification (e.g., the manufacturing of organic acids) and degradation of organ mineral compounds. Therefore, the activation of microbiological processes in the rhizosphere aimed at increasing the locomotivity and presence of metals to plants should enhance their removal from the soil with plant biomass. The biological absorption of heavy metals in soils is associated with a decrease in their mobility, due to the transition to living organisms. When absorbed by plants and alienated with the harvest, the content of metals in the soil decreases. An important form of biological absorption of metals is their assimilation by microorganisms. Some fungi and bacteria produce substances that promote the absorption of metals by cells, which reduces their toxicity (Wei et al., 2021; Rattanapolsan et al., 2021).

p0225 The toxic effect of metals begins to manifest itself with an increase in their concentration: the metabolism of organisms is disturbed through inhibition of enzyme activity in them and modification in the penetration of cell membranes.

p0230 Just as growth microorganisms on media in the attendance of high concentrations of heavy metals, an enlargement in cell size is most often observed, alike to the effect of other unfavorable chemical and physical factors on microorganisms. Some bacterial cells can take on nonspecific shapes, such as *Escherichia coli* cells taking on filamentous shape. All in all, these frustrations are linked with disunity the processes of growth and cell division. Heavy metals can also cause various other disturbances in the cell ultrastructure, such as the appearance of various inclusions, additional lipid globules, the formation of irregularly shaped mitochondria, a decrease in the number of ribosomes, and others. In photosynthetic microorganisms, under the impact of heavy metals, the content of chlorophyll in the cells decreases.

p0235 Significant changes are caused by the force of heavy metals on the cytoplasmic membrane. They are associated, first of all, with a violation of their functions, which leads to the loss of amino acids and nucleotides by cells, inhibition of transport processes, etc. Influence of heavy metals on energy and biosynthetic processes. The general mechanism of action of various heavy metals is their inhibition of respiration in microorganisms. This effect is often observed at higher concentrations of metals (4−3 M) than those that suppress the growth of microorganisms. It is believed that the suppression of respiration is associated mainly with the inhibition of the transport of substrates into the cell, or with the direct interaction of heavy metals with the components of the respiratory chain. Under the influence of heavy metals, fermentation can be inhibited, whereas photosynthesis and nitrogen fixation can be suppressed. Heavy metals also inhibit biosynthetic processes, resulting in changes in the content of basic polymers in cells. The most common effect is the inhibition of protein and RNA synthesis.

Heavy metals can be mutagenic in microorganisms, causing increased mutation rates, chromosomal aberrations, or other DNA abnormalities. Thus, heavy metals exert their toxic effect on most parts of the metabolic pathways of microorganisms (Haider et al., 2021).

p0240 The distribution of accumulated heavy metals depends on the type of microorganism and the metal itself. So mercury, cadmium, silver, uranium is sorbed mainly by bacteria and fungi on the surface of cells, only partially penetrating inside. Ions of copper, zinc, nickel, cobalt, and manganese are more often transported into the cell. The process of metal adsorption on the surface of microorganisms includes its binding to the cell wall, cytoplasmic membrane, as well as capsule substances and extracellular secretions. The interaction is connected mainly with the negative charge of these surface structures. Metal binding sites in the cell wall can be protein molecules (mercury in yeast), carboxyl groups of peptidoglycans (divalent cations in bacilli), and phosphate groups (uranium in yeast). Lead, in the form of phosphate, is adsorbed by *Citrobacter* sp. on the cell surface. Similar groups also interact with metals in the composition of the cytoplasmic membrane (Su et al., 2021).

p0245 The substance of heavy metals in cells depends on the type of microorganisms, environmental conditions and other factors. For example, the accumulation of cadmium in bacteria of the intestinal group was 0.7−89.0 mg/g of dry biomass. Lead is accumulated by cells in much greater amounts, for example, in *Azotobacter* sp. and *Micrococcus luteus*, its content reached $3.1-4.9 \times 10^2$ mg/g of dry biomass, in *Citrobacter* sp. one-third of the weight of dry cells, and some algae accumulated it up to 20%. The content of uranium in *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa* can reach 10%−15% of the weight of dry biomass. The amount of metal in the biomass of microorganisms increases almost linearly with an augmentation in its concentration in the medium, but up to a certain concentration. Inside cells, metals can be present in the form of free ions or be associated with various components.

p0250 Metals absorbed by cells can be found in various intracellular structures: membranes, ribosomes, and in the photosynthetic apparatus. Binding of heavy metals can be specific, which is typical for some cyanobacteria, algae, and fungi, as well as nonspecific. Binding specificity is determined by the presence of low molecular weight specialized proteins that are rich in cysteine and bind large amounts of heavy metals and characteristic of animal cells (metallothioneins). In bacteria, with the exception of cyanobacteria, such proteins have not been found, but other high molecular weight proteins can perform similar functions.

p0255 The development of the potential of microorganisms that mobilize metals can serve as an alternative or addition to chemical methods for increasing the mobility of heavy metals, for example, the introduction of chelating substances into the soil. Note that microbiological technologies are successfully used for enrichment of ores in the mining industry and bioremediation of radioactive waste (Chunsheng et al., 2014; Bai et al., 2022).

p0260 Under the effect of high doses of heavy metals, the number of microorganisms decreases. With technogenic pollution of ecosystems, as total of microorganisms and the content of ammonifiers and nitrifies sharply decreases, while the number of denitrifies and oligonitrifiers increases. The number of phosphate-dissolving and iron-reducing bacteria also increases, while the number of cellulose-destroying microorganisms tends to decrease. The most sensitive to soil pollution are ammonifying bacteria that use mineral nitrogen, some spore bacteria, cellulolytic bacteria, and actinomycetes. However, not in all cases, a reduction in the number of soil microorganisms was recorded. In a number of works, an increase in the total number of microflora was noted. According to some researchers, this is in connection with the death of a sensitive microorganisms and the dynamic evolution of resistant forms that make use of the energy material material of dead cells as food. There is information about the absence of significant changes in the number of microorganisms in contaminated soils (Khanna et al., 2021).

p0265 Heavy metals, acting on soil microorganisms, increasing the number of some (microscopic fungi) and reducing the number of others (bacteria, actinomycetes), lead to a change in the structure of the microbial cenosis, causing successional changes in the soil biocenosis. According to the degree of tolerance to the action of heavy metals, the main groups of soil microorganisms are arranged as follows: microscopic fungi > actinomycetes > bacteria > spore-forming bacteria.

p0270 The composition of soil microflora includes microorganisms with different requirements for nutritional conditions and energy sources. The quantitative ratios between them depend on the environmental conditions in which one or another microbial cenosis develops. For the first time, the concept of ecological and trophic groups of soil microflora was put forward by S.N. Vinogradsky, who described two functionally different groups of microorganisms: zymogenic and autochthonous. The zymogenic microflora is responsible for the decomposition of fresh plant residues, while the autochthonous microflora is responsible for the decomposition of humus. According to modern concepts, the structure of microbial cenosis, consisting of zymogenic, autochthonous, oligotrophic, and autotrophic groups of microorganisms, is continuously changing (Ka-Ot and Joshi, 2022).

s0035 11.1.4 Study of the impact of salts of heavy metals on the number of soil microorganisms

p0275 One of the main qualitative indicators of soil is microbiological activity. Soil microbiocenosis characterizes its potential fertility, the total result of biochemical processes caused by the vital activity of microorganisms. The qualitative and quantitative composition of complexes of soil microorganisms is also an important diagnostic indicator of the state of the soil, which is associated with the high sensitivity of individual representatives of microbial communities to changes in environmental conditions (Corredor et al., 2021).

p0280 The peculiarity of heavy metals to accumulation leads to a progressive change in the chemical composition of soils, and disruption of the unity of the geochemical environment and living organisms.

Egamberdieva-PESM-1633852 978-0-443-13365-7 00009

p0285 It has been established that for a long time, the intensity of accumulation of heavy metals in soils located at a closer distance from the source of emissions becomes less. Contemporaneously, the content of gross forms increases. Whereas with the distance from the source, the accumulation is more intense with an augmentation in the content of mobile forms. Soils of light granulometric composition are characterized by the maximum content of zinc, cadmium, and lead in the upper part of the profile in the humus horizon, and the minimum content of copper. For heavy loamy soils, the maximum amount of copper, zinc, and cadmium is noted in the lower horizons; lead accumulates in the upper horizons (Ma et al., 2022).

p0290 Before starting the screening of strains of fungi resistant to various concentrations of heavy metals, two soil samples were analyzed. The presence and quantitative content of elements in them, which are included in the group of heavy metals: lead, cadmium and zinc, and are elements of the 1st hazard class, were studied. An analysis of the physicochemical properties of dark chestnut soils in the Almaty region showed that, in terms of granulometric composition, the soils are light loam with a physical clay content of 20.3%−21.4%. According to the results of the study, it was revealed that the excess of MPC in the soil of heavy metals is observed in almost the entire study area. So, the content of zinc and lead does not exceed. The content of cadmium exceeds the MPC by 1.5−6.5 times (Ma et al., 2021).

p0295 Microbial communities of arable soils were studied by microbiological methods. These pooled samples were pre-contained with heavy metals, since it was necessary to know the level of soil contamination with heavy metals.

p0300 From the results obtained, it can be seen that the concentration of mobile forms of lead and zinc is significantly lower than the MPC significance (Table 11.1).

p0305 The gross content of lead, zinc is almost twice lower than the MPC. Whereas for cadmium in virgin soil, the gross and mobile forms exceed the MPC

t0010 **Table 11.1** Concentration of heavy metals in landscape soils.

Index		Pb^{2+}	Zn^{2+}	Cd^{2+}
Gross concentration (mg/kg)	1	10.4	134.8	0.8
	2	14.0	147.6	2.4
Mobile forms concentration (mg/kg)	1	1.1	0.6	0.3
	2	1.1	1.7	0.6
Mobility (%)	1	5.4	0.5	37.5
	2	4.6	1.2	25.0
MPC for gross forms in the CIS (mg/kg)[a]		130	220	2
MPC for mobile forms in the CIS (mg/kg)[a]		32.0	23.0	0.5

Note: 1—arable soil, 2—virgin soil.
[a]Letter of the Ministry of Natural Resources of the Russian Federation No. 04–25, Roskomzem No. 61–5678 dated 12/27/93 gross and mobile forms.

significance by 18%. In arable soil, these figures are almost two times lower than the MPC significance.

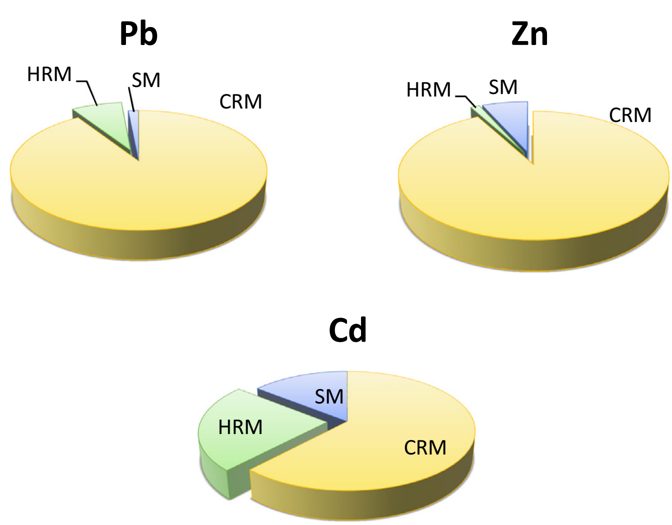
p0310 Heavy metals are currently among the most widespread environmental pollutants that penetrate into living cells, disrupt their vital functions, manifesting their toxic effect in the form of ions. It is known that microorganisms are currently persistent to almost all heavy metals (Alothman et al., 2019).

s0040 11.1.5 Screening for fungi sustained to various concentrations of heavy metals

p0315 Fifty-three cultures isolated at a low content of heavy metal in the medium were used for further experiments, subculturing them on media containing heavy metal in an amount of 1 mM.

p0320 As a result, as can be seen in Fig. 11.1, the studied cultures were divided into three large groups.

p0325 Representatives of the first group were sensitive to heavy metal, the second group were conditionally resistant (CRM), and the third group had well-pronounced heavy metal resistance (HRM). The first group of microorganisms sensitive to the action of heavy metals included cultures: *Rhodotorula* sp. *A1 Talaromyces pinophilus 3 R, Metarhizium* sp. *An2 Cryptococcus al. K3, Penicillium restrictum 8 C, Penicillium bilaiae 11 C, Talaromyces pinophilus T14, Aspergillus alliaceus T6*, and *Mortierella alpine EFW1*. These strains did not grow on the nutrient medium, probably due to their high sensitivity to heavy

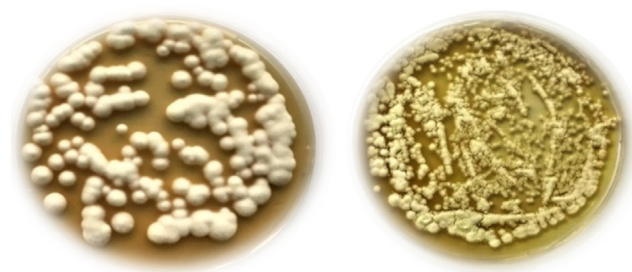


f0010 **FIGURE 11.1**

Sensitivity of fungi to heavy metal.

metal action. To the second—*Fus. culmorum Fus 2, Trichoderma* sp. *D3, Trichoderma* sp. *D1, Penicillium bilaiae Pb14, Aspergillus* sp. *Asp 5, Penicillium* sp. *EFW2, Penicillium commune T1, Aspergillus* sp. *AC4, Rhodotorula* sp. *EY5, Aureobasidium* sp. *C7, Metschnicowia* sp. *MP1, Rhodotorula* sp. *A1, Penicillium citrinum EF2, F. solani, Trichoderma pseudokoningii D4*. The third group includes fungal strains of *Beauveria* sp. *T15, Beauveria* sp. *T7*, and *yeast strains: Metschnicowia* sp. *MP2, Rhodotorula* sp. *RH, Rhodotorula* sp. *MK1*.

p0330 When selecting fungi resistant to heavy metals, five strains had the maximum significance of CFU/mL. Cultures with multiple resistance to the toxic effects of heavy metals, as shown in Figure 3.4, strains of fungi *Beauveria* sp. *T15, Beauveria* sp. *T7*, and yeast strains in Figure 5.6: *Metschnicowia* sp. *MP2, Rhodotorula* sp. *RH, Rhodotorula* sp. *MK1*. CFU indicators for them ranged from 1.1×10^5 to 3.5×10^6. The strain *Rhodotorula* sp. *MK1* possessed the maximum resistance (Figs. 11.2–11.5).



1 2

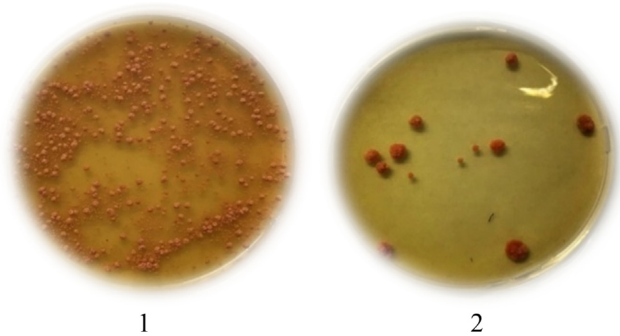
f0015 **FIGURE 11.2**

Colonies of *Beauveria* sp. *T7* and *Beauveria* sp. *T15* on a medium with cadmium Cd^{2+}. 1—*Beauveria* sp. T7, 2—*Beauveria* sp. T15.

1 2

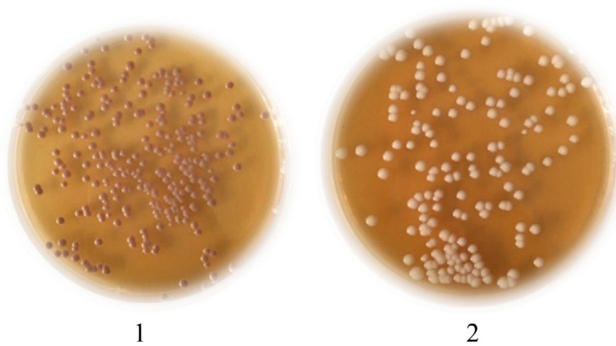
f0020 **FIGURE 11.3**

Colonies of *Beauveria* sp. T7 and *Beauveria* sp. T15 on a medium with zinc Zn^{2+}. 1—*Beauveria* sp. T7, 2—*Beauveria* sp. T15.



f0025 **FIGURE 11.4**

Colonies of *Rhodotorula* sp. MK on a medium with metals. 1—*Rhodotorula* sp. МК1 on a medium with lead Pb, 2—*Rhodotorula* sp. МК on a medium with zinc.



f0030 **FIGURE 11.5**

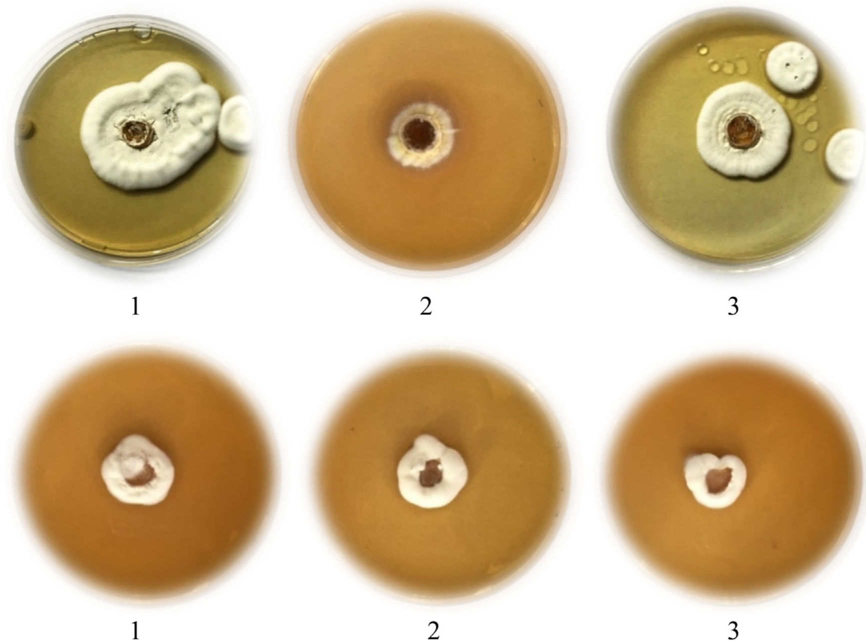
Colonies *Metschnicowia* sp. MP2 on a medium with metals. 1—*Metschnicowia* sp. MP2 on a medium with lead Pb, 2—*Metschnicowia* sp. MP2 on a medium with zinc Zn.

p0335 Heavy metals can greatly reduce the biological activity of soil. With the help of microorganisms, it is possible to convert heavy metal ions into organometallic compounds, where they will not show their toxicity.

p0340 To select microorganisms resistant to heavy metals, fungal and yeast cultures were sown by the method of agar blocks on a dense Saburo medium with various metal concentrations.

p0345 Fig. 11.6 depicts an exhibition of strains of filamentous fungi *Beauveria* sp. characterized by maximum resistance to the studied metals. T15 and *Beauveria* sp. T7. The tolerance index of these strains for zinc, lead, and cadmium ranged from 0.90 to 1.16.

p0350 The studied tolerance index showed in Figure 7.8 that the isolated strains have multiple tolerance to heavy metal. Of the studied mushrooms, 1 showed a



f0035 **FIGURE 11.6**

Tolerance of filamentous fungi to the action of heavy metal (agar block method). 1—*Beauveria* sp. T15 on Saburo, 2—*Beauveria* sp. T15 on a medium with zinc (Zn^{2+}), 3—*Beauveria* sp. T15 on a medium with cadmium (Cd^{2+}). 1—*Beauveria* sp. T7 on Saburo (Control), 2—*Beauveria* sp. T7 on a medium with zinc (Zn^{2+}), 3—*Beauveria* sp. T7 on a medium with cadmium (Cd^{2+}).

sky-high tolerance—this is *Beauveria* sp. *T15* with IT 1.12. The four manifestations of high tolerance are *Beauveria* sp. *T7 with IT 0.91; Rhodotorula* sp. *MK1* and *Rhodotorula* sp. *RH* with IT 0.88 and *Metshnicowia* sp. *MP2* with IT 0.90. For Zn and Pb metals, the tolerance index of strains is from 0.08 to 0.19 and 0.03 to 0.015.

p0355 It can be seen from the obtained results that some micromycetes, such as *Beauveria* sp. *T15* and *Beauveria* sp. T7 showed multiple tolerance to heavy metal. The strains showed high tolerance towards cadmium.

p0360 Cadmium belongs to the first hazard class among heavy metals. The study of microorganisms resistant to cadmium is extremely important to refine the efficacy of the phytoremediation process.

p0365 To confirm the resistance to heavy metals of the studied cultures, they were grown in liquid Saburo nutrient medium with the addition of cadmium salts Cd^{2+} at a concentration of 1:2 and 3 mm.

p0370 After cultivation for 96 hours at a temperature of 25°C, the biomass of microorganisms was determined by the gravimetric method.

p0375 From the results of the experiment, it can be seen that among the five explored strains of micromycetes, the fungus *Beauveria* sp. T15 has the highest resistance to cadmium.

p0380 The addition of cadmium ions to the medium has a significant effect on the change in the number of cells in the culture: the higher the concentration of cadmium, the more noticeable its negative effect on the growth of micromycete cells.

p0385 As can be seen from, the studied cultures are resistant to high concentrations of cadmium in the medium (3 mM cadmium).

p0390 In relation to cadmium, the strain *Beauveria* sp. T15 was stable with biomasses of 1.52 and 1.51 mg/mL at a cadmium concentration of 1 mM.

p0395 When studying the effect of cadmium ions on yeast growth, it was demonstrated that resistant strains are *Rhodotorula* sp. MK, *Rhodotorula* sp. RH and Metschnicowia sp. MP2 (Figs. 11.5−11.7). *Rhodotorula* sp. *MK1, Rhodotorula* sp. RH and *Metschnicowia* sp. MP2 are capable to grow at a cadmium concentration in the medium up to 339.36 mcg/mL, while the amount of biomass for the *Rhodotorula* sp. MK1 varied within 0.16−1.33 mg/mL, for *Metschnicowia* sp. MP2 0.15−0.74 mg/mL, for *Rhodotorula* sp. RH 0.13−0.75 mg / mL.

p0400 As a result of studying the growth of fungi at varied concentrations of cadmium were selected strains of *Beauveria* sp. T7, *Beauveria* sp. T15 and yeast strains: *Rhodotorula* sp. MK1, *Metschnicowia* sp. MP2, *Rhodotorula* sp. RH.

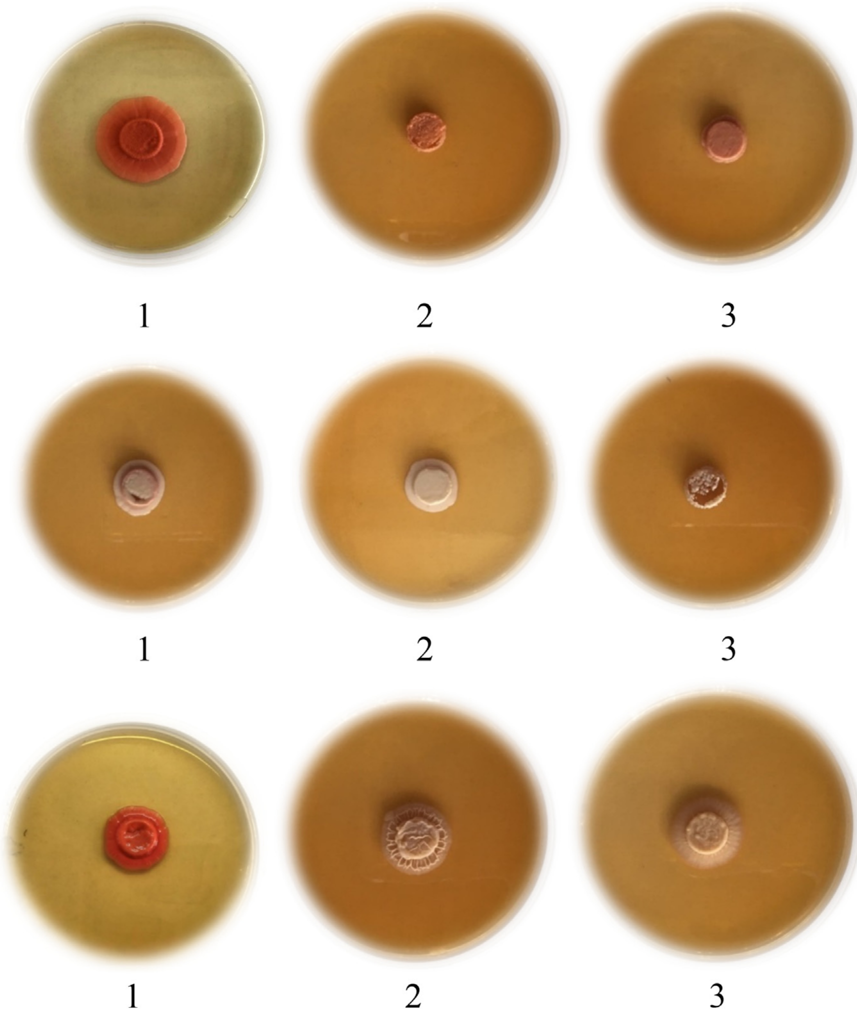
s0045 11.1.6 Determination of MTC and MIC of cadmium for the studied strains of micromycetes

p0405 For strains that showed resistance to cadmium, the following indicators of resistance were determined: maximum tolerant concentration (MTC) and minimum inhibitory concentration (MIC). While it is critical to find fungi with a high ability to survive high concentrations of heavy metals, these fungi should be able to conform and grow rapidly at given metal concentrations. This means that the faster fungi can adapt to a polluted environment and develop their colonies, the more profitable it will be to use them (Alothman et al., 2019; An et al., 2015).

p0410 Maximum tolerant concentration (MTC) is an indicator of culture resistance to the action of a metal, equal to its maximum concentration at which culture growth is observed (Aryal, 2020).

p0415 The resistance of fungi to heavy metal ions was determined by studying the MTC. Plates with Saburo were prepared with metal (Cd^{2+}) at different concentrations (from 50 to 500 mcg/mL). The plates were incubated at 25°C ± 1°C for 7−14 days.

p0420 The strain *Beauveria* sp. T15−2400 mcg/mL. The MIC of cadmium was somewhat lower in strains of *Beauveria* sp. T7−1400 mcg/mL and *Rhodotorula* sp. MK1−1460 mcg/mL. In strains of *Rhodotorula* sp. *RH*—430 mcg/mL and *Metschnicowia* sp. MP2−290 mcg /mL.



f0040 **FIGURE 11.7**

Tolerance of yeast strains to heavy metal exposure (agar block method). 1—*Rhodotorula* sp. RH on Saburo (Control), 2—*Rhodotorula* sp. RH on a medium with lead (Pb^{2+}), 3—*Rhodotorula sp*. RH on a medium on a medium with cadmium (Cd^{2+}). 1—*Metshnicowia* sp. MP2 on Saburo (Control), 2—*Metshnicowia* sp. MP2 on a medium with zinc (Zn^{2+}), *Metshnicowia* sp. MP2 on a medium with cadmium (Cd^{2+}). 1—*Rhodotorula* sp. MK1 on Saburo (Control), 2—*Rhodotorula* sp. MK1 on a medium with lead (Pb^{2+}), 3—*Rhodotorula* sp. MK1 on a medium with cadmium (Cd^{2+}).

p0425 The level of individual metal opposition of the strains was assessed by determining the Minimum Inhibitory Concentration (MIC) of the metal salt (Aryal, 2020; Singh et al., 2021).

p0430 The resistance of fungi to heavy metal ions was determined by studying the MIC. Saburo plates were prepared with metal (Cd^{2+}) at different concentrations (from 50 to 500 mcg/mL). The plates were incubated at 25°C ± 1°C for 7−14 days.

p0435 When determining the MIC salt of cadmium for metal-resistant strains of fungi, differences were established in the average significance and the range of MIC significance of individual fungi. The mean MIC significance of these metals differed statistically significantly ($P < 0.05$).

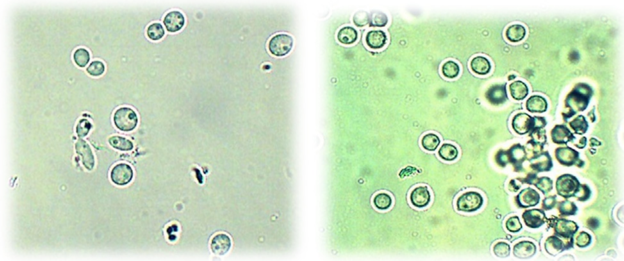
p0440 When determining the MIC salt of cadmium for metal-resistant strains of micromycetes, differences were established in the average significance and the range of MIC significance of individual micromycetes. The mean MIC significance of these metals differed statistically significantly ($P < 0.05$).

p0445 The highest MIC significance for the existence of cadmium in the medium was shown by the strain *Beauveria* sp. T15−2450 mcg/mL. The MIC of cadmium was somewhat lower in strains of *Beauveria* sp. T7−1450 mcg/mL and *Rhodotorula* sp. MK1−1500 mcg/mL. In strains of *Rhodotorula* sp. RH—480 mcg/mL and *Metschnicowia* sp. MP2−320 mcg/mL.

p0450 MIC significance of 0.328 mM for filamentous fungi and 1 (mg/L) for *Aspergillus, Penicillium*, and *Fusarium* have been reported in the literature. In a study by Kondratenko in 2007, MIC significance for 3000, 4000, and 5000 (mg/L) Cd were recorded for *Rhizopus* sp*., Terichoderma*, and *Aspergillus*, respectively. These data on VPK are relatively close to the significance we observed. When growing on media with metal at a concentration of 100 mcg/mL, changes in the macro- and micromorphology of fungi were observed compared to the control. The changes that occur in the cells of microorganisms are primarily associated with the morphology of the cells. When microorganisms are cultivated on media in the presence of high concentrations of heavy metals, an increase in cell size is most often observed, similar to the effect of other unfavorable chemical and physical factors on microorganisms. Some bacterial cells can take on nonspecific shapes, such as *E. coli* cells taking on filamentous shapes. Generally, these frustrations are connected with uncoupling of cell growth and division processes. Yeast resistance to the toxicant effect of heavy metals relies on either morphological and physiological characteristics of the cell (Singh et al., 2021).

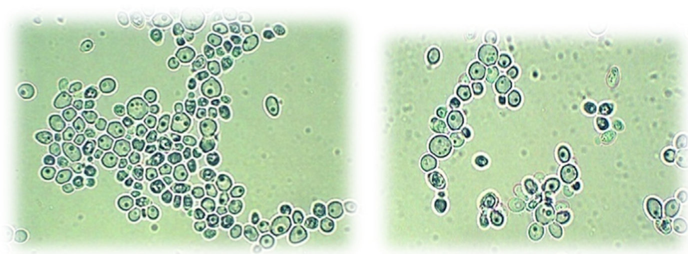
p0455 When conducting microscopy of the studied strains in Figs. 11.8−11.12, it was found that the presence of cadmium in the medium probably enlarged the permeability of the cell membrane, which led to the formation of spherical cells. On the example of *Metschnicowia* sp. MP2 it has been shown that accumulation of Cd^{2+} is accompanied by cell membrane mineralization. Cd^{2+} toxicity in this case manifested itself in a gradual change in cell morphology, such as shortening of trichomes and an increase in cell size.

p0460 Therefore, as per the literature data, it is known that there are some gears of protection of microorganisms from the toxic effects of heavy metals (Karn et al., 2021).



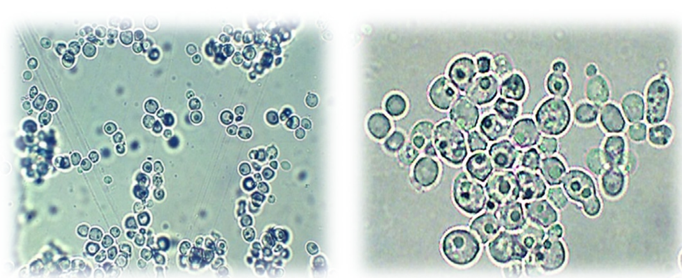
f0045 **FIGURE 11.8**

Culture of *Rhodotorula* sp. MK1 on a medium with cadmium Cd^{2+} in concentration of 3 mM (magn. × 1500).



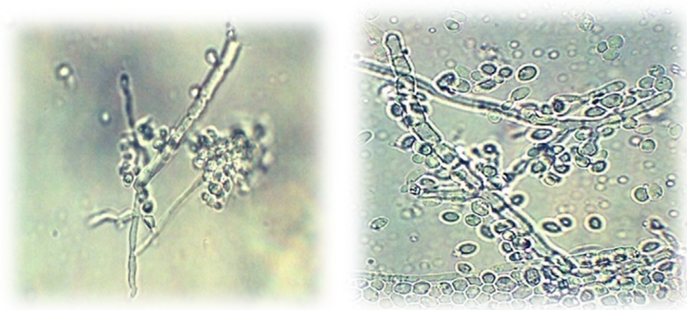
f0050 **FIGURE 11.9**

Culture of *Metschnicowia* sp. MP2 on a medium with cadmium Cd^{2+} in concentration of 1 mM (magn. × 1500).



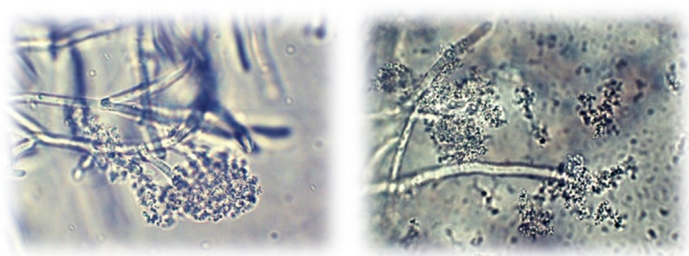
f0055 **FIGURE 11.10**

Culture of *Rhodotorula* sp. *Rh* on a medium with cadmium Cd^{2+} in concentration of 3 mM (mag. × 1,500).



f0060 **FIGURE 11.11**

Culture of *Beauveria* sp. T7 on a medium with cadmium Cd^{2+} in concentration of 3 mM (magn. × 1,500).



f0065 **FIGURE 11.12**

Culture of *Beauveria* sp. T15 on a medium with cadmium Cd^{2+} in concentration of 3 mM (magn. × 1,500).

p0465 The fundamental mechanisms are the ability of microorganisms to release into the environment substances that form insoluble complexes with heavy metal ions and the binding of heavy metals in the cell cytoplasm.

p0470 The intensity of a metal can manipulate the morphology and physiology of cells in varied ways. When cultivating yeast on dense media containing cadmium at a concentration close to the MIC, a long growth retardation was seen, after which growth and congestion of biomass by the culture occurred, and the higher the concentration of the metal, the more different (were lower) these indicators were from the control significance. This corresponds to similar studies carried out with the study of cadmium uptake and, as a reaction to this metal, the change in the duration of the lag phase during the cultivation of *Rhodotorula* sp. Y11 (Karn et al., 2021).

p0475 In Figs. 11.13 and 11.14, colonies of strains of *Beauveria* sp. T15 and *Beauveria* sp. T7 on the medium with cadmium become more dense, with drops

f0070 **FIGURE 11.13**

Colonies of *Beauveria* sp. T15 on medium with different concentrations of Cd^{2+}. 1—Culture of *Beauveria* sp. T15 on control medium without metal, 2—Culture of *Beauveria* sp. T15 with a concentration of Cd^{2+} 100 mcg/mL, 3—with a concentration of Cd^{2+} 2400 mcg/mL.

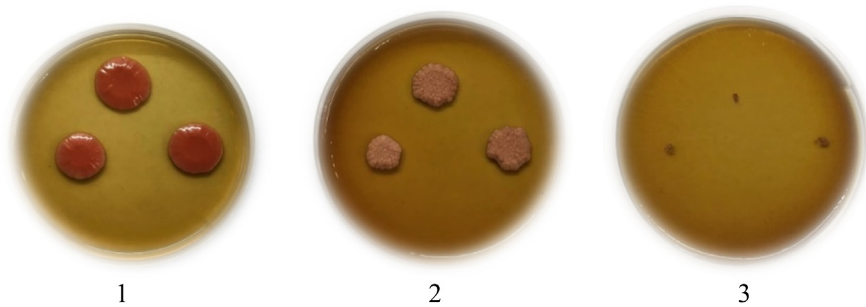
f0075 **FIGURE 11.14**

Colonies of *Beauveria* sp. T7 on medium with different concentrations of Cd^{2+}. 1—Colonies of the strain *Beauveria* sp. T7 1-on the control on the medium without metal, 2—Colonies of the strain *Beauveria* sp. T7 with a concentration of Cd^{2+} 100 mcg/mL, 3—Colonies of the strain *Beauveria* sp. T7 with a concentration of Cd^{2+} 1,400 mcg/mL.

of exudate, have a pronounced substrate mycelium growing into the agar, which leads to cracking of the agar. There was no color change.

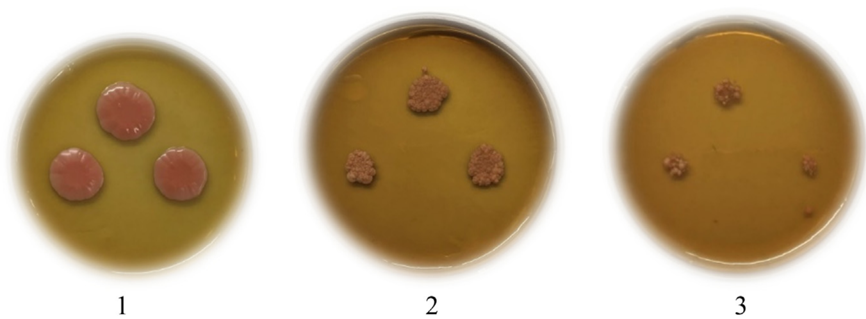
p0480 Fig. 11.15 shows colonies of *Rhodotorula* sp. MK1—in the control, the colonies are bright pink, smooth, shiny, glossy, the edges are even. On the medium with metals, the colony turns pale, to slightly pink, becomes dull and bumpy, with pronounced pigmentation of the colony.

p0485 Fig. 11.16 shows colonies of *Rhodotorula* sp. RH—on the medium without metal, the colonies are slightly pink, matte, even. On a medium with metals, they grow in the form of small individual tuberous colonies, and fade significantly.



f0080 **FIGURE 11.15**

Colonies of *Rhodotorula* sp. MA with different concentrations of Cd^{2+}. 1—Colonies of the strain *Rhodotorula* sp. MK on the control medium without metal, 2—Colonies of the strain *Rhodotorula* sp. MK with a concentration of Cd^{2+} 100 mcg/mL, 3—Colonies of the strain *Rhodotorula* sp. MK concentration of Cd^{2+} 1,460 mcg/mL.



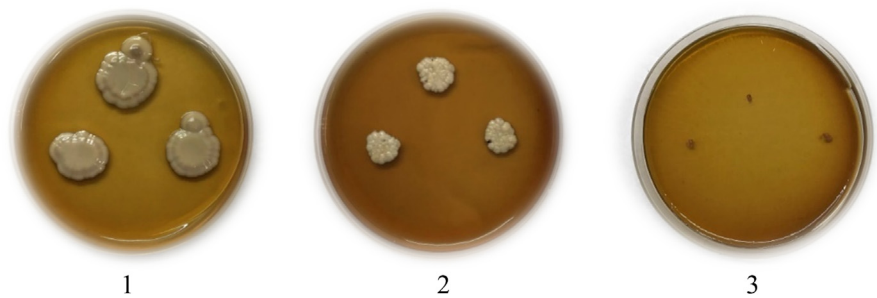
f0085 **FIGURE 11.16**

Colonies of *Rhodotorula* sp. *Rh* on a medium with different concentrations of Cd^{2+}. 1—Colonies of the strain *Rhodotorula* sp. Rh on the control medium without metal, 2—Colonies of the strain *Rhodotorula* sp. Rh with a concentration of Cd^{2+} 100 mcg/mL, 3—Colonies of the strain *Rhodotorula* sp. Rh concentration Cd^{2+} 430 mcg/mL.

p0490 Fig. 11.17 shows colonies of *Metschnicowia* sp. MP2—in the control, the colonies are white-pink with the release of pigment into the medium, shiny, smooth edges. On the medium with metal, the colonies become bumpy, the pigment is released into the medium only by individual colonies, and it is not as bright as in the control.

p0495 For yeast cultures, as seen in Figs. 11.15–11.17, colony fading is characteristic, the color becomes faded, as shown in the figures.

p0500 The operation of metal adsorption on the surface of microorganisms includes its binding to the cell wall, cytoplasmic membrane, as well as capsule substances



f0090 **FIGURE 11.17**

Colonies of the *Metschnicowia* sp. MP2 strain on a medium with various concentrations of Cd^{2+}. 1—Colonies of the *Metschnicowia* sp. MP2 strain on a control medium without metal, 2—Colonies of the *Metschnicowia* sp. MP2 strain with a Cd^{2+} concentration of 100 mcg/mL, 3—Colonies of the *Metschnicowia* sp. MP2 strain, Cd^{2+} concentration of 290 mcg/mL.

and extracellular secretions. The interaction is connected mainly with the negative charge of these surface structures. Metal binding sites in the cell wall can be protein molecules (mercury in yeast), carboxyl groups of peptidoglycans (divalent cations in bacilli), phosphate groups (uranium in yeast). Lead, in the form of phosphate, is adsorbed by *Citrobacter* sp. on the cell surface. Similar groups also interact with metals in the composition of the cytoplasmic membrane (Shylla et al., 2021).

s0050 11.2 Conclusion

p0505 Soil contamination with heavy metals is one of current environmental problems due to the increasing amounts of metals entering the environment. The composition of such pollutants is extremely diverse, it changes in the process of the emergence of new industries and the improvement of existing ones. Methods for removing heavy metals from the encirclement can be separated into two groups: (1) biotic methods, which are based on the accumulation of heavy metals by plants or microorganisms, and (2) abiotic methods, which are based on such physicochemical processes as precipitation, adsorption, etc. Compared to physicochemical methods for removing heavy metals from the environment, the use of biological methods is considered cost-effective and ecological safe. Several mechanisms of bacterial resistance to heavy metal ions are known: extracellular barrier, active transport of metal ions from the cell, extracellular binding, intracellular binding, and reduction of metal ions.

p0510 As a result of the studies, it was found that the studied strains have the capability to accumulate cadmium ions. The process of cadmium uptake by fungi is complex and is carried out through passive binding by cellular structures and active transport. The data obtained allow us to note that a significant part of the process of absorption of cadmium ions by micromycete cells is provided by active transport. The toxicity against micromycetes, heavy metals form a series: $Cd^{2+} > Pb^{2+} > Zn^{2+}$. The CFU/mL for lead was $(12.6-37.1) \times 10^5$, for zinc $(16.6-33.2) \times 10^5$, and for cadmium $(1.7-3.6) \times 10^5$.

p0515 The strains of *Beauveria* sp. were characterized by the maximum resistance to the studied metals. T15 and *Beauveria* sp. T7. The tolerance index for zinc, lead, and cadmium ranged from 0.90 to 1.16.

p0520 The significance of MTC and MIC of cadmium were determined, which were for the following strains: *Beauveria* sp. T15 0.6593 and 0.6730 mcg/mL; *Rhodotorula* sp. MK1 0.4065 and 0.4150 mcg/mL; *Beauveria* sp. T7 0.3846 and 0.3983 mcg/mL; *Metschnicowia* sp. MP2 0.0793 and 0.0879 mcg/mL, *Rhodotorula* sp. RH 0.1181 and 0.1318 mcg/mL.

p0525 The maximum extraction of cadmium from the nutrient medium was noted for cultures of *Beauveria* sp. T7 and *Rhodotorula* sp. MK1 with an absorption rate of 58.7% and 48.5%, respectively. *Rhodotorula* sp. MK1, *Beauveria* sp. T7, and *Rhodotorula* sp. RH2 accumulated 11.91; 11.01, and 8.87 mg/g cadmium, respectively. When studying the localization of cadmium (intracellular and extracellular), it was shown that most cultures accumulate intracellular cadmium to a greater extent ($I = 61.8\%-80.4\%$).

p0530 The results of the survey can promote the efficacious use of the possibility of microorganisms to expand the response of plants to stress agents, which can become the basis for the elaboration of technology for growing plants under the action of heavy metals.

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