
EXPERIMENTAL
ARTICLES

Employment of Rhizobacteria for the Inoculation of Barley Plants Cultivated in Soil Contaminated with Lead and Cadmium

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Abstract—In laboratory experiments, the rhizobacteria *Azospirillum lipoferum* 137, *Arthrobacter mysorens* 7, *Agrobacterium radiobacter* 10, and *Flavobacterium* sp. L30 were found to have a relatively high resistance to the toxic heavy metals lead and cadmium (except that strain L30 was found to be sensitive to Cd). When introduced by means of seed bacterization, the heavy metal-resistant strains actively colonized the rhizosphere of barley plants cultivated in uncontaminated and contaminated soils. In both pot and field experiments, seed bacterization improved the growth of barley plants and the uptake of nutrient elements from soil contaminated with Pb and Cd. The bacterization also prevented the accumulation of Pb and Cd in barley plants, thereby mitigating the toxic effect of these heavy metals on the plants.

Key words: rhizobacteria, cadmium, lead, rhizosphere, barley.

The contamination of soil with toxic heavy metals, such as lead and cadmium, leads to their accumulation in agricultural products, decreases the consumption of nutrient elements by crop plants, and diminishes the crop yield [1, 2]. The accumulation of heavy metals in soil decreases the species diversity of soil microflora and inhibits many microbiological processes [3]. At the same time, some microorganisms are fairly resistant to heavy metals and can influence their mobility in the surrounding soil by changing its pH and redox potential. Moreover, such microorganisms can absorb and accumulate some heavy metals by producing chelating agents and siderophores [4].

There is evidence that rhizobacteria can play an essential role in the resistance of plants to stress induced by heavy metals. For instance, wheat plants accumulate less Cd [5] and Pb [6] when they are cultivated in nonsterile soil than when they are cultivated in sterile soil. The inoculation of rape and brown mustard plants with rhizobacteria was found to enhance the resistance of the plants to Ni, Pb, Zn, and Cd [7, 8].

Our earlier experiments with the rhizobacteria *Azospirillum lipoferum* 137, *Arthrobacter mysorens* 7, *Agrobacterium radiobacter* 10, and *Flavobacterium* sp. L30 showed that they stimulate root growth and increase the content of nutrient elements in barley seedlings cultivated in hydroponic culture in the presence of

cadmium [9]. This prompted us to study in more detail the resistance of these rhizobacteria to Pb and Cd and to investigate the effect of the rhizobacteria on barley plants cultivated in soil contaminated with these heavy metals.

MATERIALS AND METHODS

Experiments were carried out with the industrial rhizobacterial strains *Azospirillum lipoferum* 137, *Arthrobacter mysorens* 7, *Agrobacterium radiobacter* 10, and *Flavobacterium* sp. L30, which were obtained from L.F. Vasyuk and V.F. Pavlova, All-Russia Research Institute of Agricultural Microbiology.

The heavy metal resistance of the rhizobacteria was evaluated by estimating their growth at 28°C for 5 days on agar RTM medium (pH 6.8), which contained (g/l) glucose, 1; sucrose, 1; peptone, 1; yeast extract, 1; NH₄NO₃, 0.5; MgSO₄, 0.2; NaCl, 0.1; CaCl₂, 0.02; and FeCl₃, 0.01. The medium was supplemented with one of the heavy metal salts—Pb(C₂H₃O₂)₂, CdCl₂, CuCl₂, NiCl₂, or ZnCl₂—taken at concentrations from 0 to 2 mM. The accumulation of Cd by the rhizobacteria was studied by cultivating them at 28°C for 4 days in liquid RTM medium supplemented with 50 μM CdCl₂ (these experiments were performed in quadruplicate). The culture was centrifuged at 10000 g for 15 min. The sedimented cells were washed twice and resuspended in distilled water. The optical density of the cell suspen-

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Table 1. The heavy metal tolerance of rhizobacteria grown on agar medium

Strain	Heavy metal concentration in the medium, μM				
	Pb	Cd	Cu	Ni	Zn
<i>Az. lipoferum</i> 137	200/700	30/350	50/150	150/400	400/1000
<i>Ar. mysorens</i> 7	400/1000	50/600	200/700	50/250	500/1500
<i>Ag. radiobacter</i> 10	200/800	60/500	100/450	150/500	150/800
<i>Flavobacterium</i> sp. L30	100/500	2/10	70/300	25/200	400/1000

Note: The minimal inhibitory and lethal concentrations of heavy metals are shown on the left and on the right of the solidus symbols, respectively.

sion was measured at 540 nm with a KFK-2UHL photoelectrocolorimeter. Aliquots of the bacterial suspension and the culture liquid (supernatant after the first centrifugation) were evaporated at 95°C to dryness. The residues were dissolved in concentrated H_2SO_4 , again evaporated to dryness, and then dissolved in 10% HNO_3 . The content of Cd in the samples was determined by using a Perkin-Elmer 403 atomic absorption spectrophotometer equipped with an HGA-74 graphite cell for electrothermal atomization. The partition coefficient of Cd between bacterial cells and the medium was calculated as the ratio of the Cd concentrations (expressed in $\mu\text{g Cd/mg}$) in the dry biomass and the culture liquid.

In pot experiments, the barley cultivar Tselinnyi-5 seedlings (six per each of the six replicate pots with 2.5 kg of soil) were cultivated in soddy podzolic soil containing (mg/kg) humus, 13000; total N, 1500; mobile P, 400; mobile K, 190; total Pb, 17; total Cd, 0.8 (pH 6.0). The soil was fertilized with (mg/kg) NH_4NO_3 , 300; K_2HPO_4 , 250; KCl, 125; MgSO_4 , 60; H_3BO_3 , 3; MnSO_4 , 3; ZnSO_4 , 3; and Na_2MoO_4 , 1.5. To study the effect of heavy metal contamination, the soil was supplemented with $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$ to a Pb content of either 100 or 500 mg/kg soil or with $\text{Cd}(\text{NO}_3)_2$ to a Cd content of either 30 or 75 mg Cd/kg soil. After adding the fertilizer and heavy metal salts, the soil was moistened, kept in the pots for 10 days, and then sowed with barley seeds, which were preliminarily surface-sterilized with a hydrogen peroxide–ethanol (1 : 1) mixture for 3 min. The sown seeds were bacterized with aqueous suspensions of rhizobacteria in an amount of 10^7 cells/seed. The rhizobacteria used for bacterization were resistant to rifampicin and streptomycin at concentrations of 40 and 500 mg/l, respectively [10]. The barley seedlings were cultivated for 40 days to the heading stage. The survival rate of rhizobacteria in the barley rhizoplane was determined as described earlier [10].

Field experiments with the barley cultivar Abava were carried out in 1994 (experiment 1) and 1995 (experiment 2) at the All-Russia Research Institute for Agriculture on Reclaimed Lands on quadruplicate test plots with an area of 1×1.6 m each. The ground of the test plots was a slightly loamy soddy podzolic soil. During experiments 1 and 2, the soil contained, respec-

tively, 18000 and 22000 mg/kg humus, 14 and 12 mg/kg nitrate N, 29 and 23 mg/kg ammonium N, 130 and 110 mg/kg mobile P, 192 and 195 mg/kg mobile K, and had pH 5.1 and 5.8. The soil of the arable horizon (0–20 cm), was fertilized with N30P80K90. To study the effect of heavy metal contamination, the soil was supplemented with $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$ to a Pb content of either 100 or 300 mg/kg or with $\text{Cd}(\text{NO}_3)_2$ to a Cd content of either 5 or 15 mg/kg soil. The heavy metal salts were added to the soil 40 days before sowing. Barley seeds used for sowing were inoculated with the commercial biopreparations azorisin (*Az. lipoferum* 137), mysorin (*Ar. mysorens* 7), agrofил (*Ag. radiobacter* 10), and flavobacterin (*Flavobacterium* sp. L30) in amounts of 1.5 g/kg seeds [11]. The amount of the respective rhizobacteria in the biopreparations, which were produced at the All-Russia Research Institute of Agricultural Microbiology on the basis of peat sterilized by gamma radiation, was no smaller than 10^9 cells/g peat. The content of P, K, S, Ca, Pb, and Cd in barley plants was determined by X-ray fluorescence analysis with a BDPK-1425 detector and an AI1029-95 multichannel amplitude analyzer.

Experimental data were statistically processed by the conventional method of standard deviations and bifactorial variance analysis performed with the aid of the Statistica V-5 program (Statsoft, the United States).

RESULTS AND DISCUSSION

Laboratory experiments showed that all the strains, except for the Cd-sensitive strain *Flavobacterium* sp. L30, are able to grow in the presence of high Pb, Cd, Cu, Ni, and Zn concentrations (Table 1) and hence can be considered tolerant to these heavy metals [12, 13]. The rhizobacteria *Ar. mysorens* 7 and *Ag. radiobacter* 10 exhibited the highest tolerance to Cd and Pb.

In pot experiments, the detrimental effect of Pb on the survival of *Az. lipoferum* 137 and *Flavobacterium* sp. L30 cells in the barley rhizoplane was recorded on the 25th and 40th days of plant cultivation (Fig. 1). Cadmium ions decreased the survival rate of these rhizobacteria in the barley rhizoplane at the beginning of the vegetative period of plants. During further cultivation, *Az. lipoferum* 137 was found to adapt to unfav-

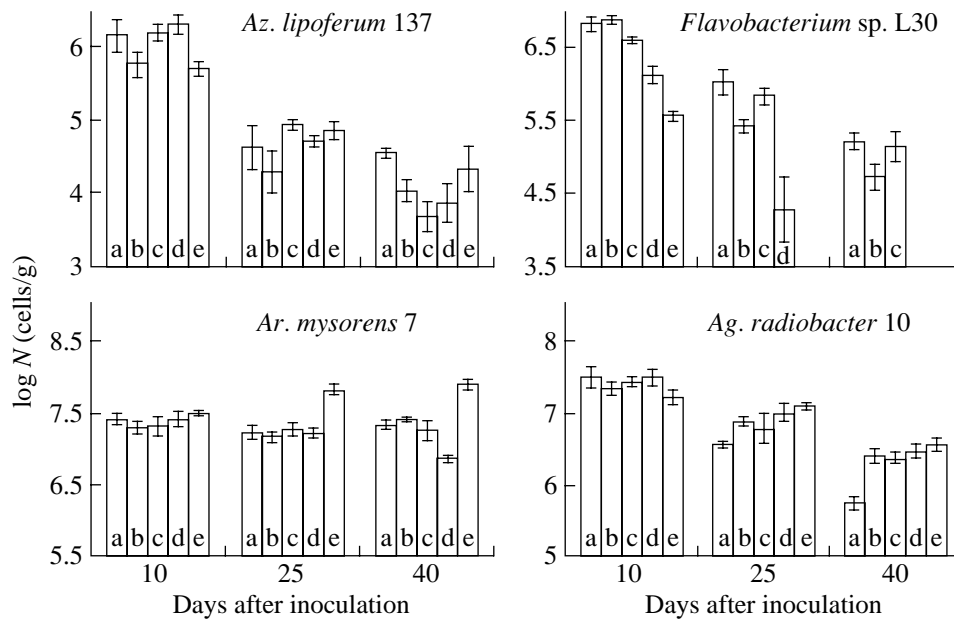


Fig. 1. The number (N) of introduced rhizobacteria in the rhizoplane of the barley plants cultivated in pots with (a) the uncontaminated soil and soil contaminated with (b) 100 mg Pb, (c) 500 mg Pb, (d) 30 mg Cd, and (e) 75 mg Cd per kg soil. The error bars present standard deviations.

avorable environmental conditions, whereas *Flavobacterium* sp. L30 was found to be eliminated from the barley rhizoplane. The colonization of barley roots with the heavy metal-resistant strains *Ar. mysorens* 7 and *Ag. radiobacter* 10 did not decrease in the presence of Pb and Cd ions and sometimes was even better than in their absence. This finding can be accounted for by the fact that the competitive ability of indigenous rhizosphere bacteria is suppressed by Pb and Cd to a greater degree than the competitive ability of the introduced rhizobacteria, which are resistant to these heavy metals. This explanation is consistent with the earlier observation that the population of the rifampicin-resistant strain *Az. lipoferum* 137 in the barley rhizoplane increases when indigenous rhizoplane microflora is inhibited by rifampicin [14].

The contamination of the soil with Pb and Cd led to a decrease in the biomass of grown barley plants and caused the accumulation of these heavy metals in the plant tissues (Table 2). At the same time, the contamination of the soil with Pb reduced the content of the nutrient elements S and Ca in the grown barley plants, whereas Cd contamination diminished the content of S and K. The introduction of *Az. lipoferum* 137 increased the biomass of grown barley plants irrespective of whether they were cultivated in the uncontaminated soil or in the soil contaminated with cadmium (Table 2). The introduction of *Ar. mysorens* 7 exerted a beneficial effect on the growth of barley plants in the uncontaminated soil, and the introduction of *Ag. radiobacter* 10 stimulated the growth of barley plants in the soil contaminated with 75 mg Cd/kg. The introduction of the rhizobacteria augmented the content of P and K in the

barley plants grown in the soil contaminated with lead. In the presence of 100 mg Pb/kg soil, the introduction of *Az. lipoferum* 137 enhanced the content of S and Ca in barley shoots, whereas *Ar. mysorens* 7 increased the content of Ca. In the case of Cd contamination, the inoculation of *Az. lipoferum* 137 augmented the content of P and S in the barley tissues, and the introduction of *Ar. mysorens* 7 was found to increase the content of S. When the soil used for experiments was contaminated with 500 mg Pb/kg, the introduction of *Ag. radiobacter* 10 and *Flavobacterium* sp. L30 could not prevent the accumulation of Pb by the growing barley plants, but Pb did not exert any detrimental effect on the growth of barley plants in the presence of these rhizobacteria. At the same time, the introduction of either of the two strains prevented the accumulation of Cd ions by the barley plants that were cultivated in the soil contaminated with 75 mg Cd/kg.

In the field experiment 1, the contamination of the plot soil with lead did not influence the barley grain yield, nor did it change the content of the nutrient elements P, K, S, and Ca and the contaminant Pb in the grain (Table 3). A statistically significant stimulation of the growth of barley plants in response to treatment with the rhizobacterial biopreparations was observed only for mysorin, which augmented (by 42%) the straw biomass of the barley plants cultivated in the plot soil contaminated with 100 mg Pb/kg (data not presented). The bacterization of barley diminished the accumulation of Pb in the grain, depending on the type of biopreparation used and the concentration of Pb in the soil. For instance, azorisin reduced the accumulation of Pb in the grain irrespective of whether the soil was con-

Table 2. The bifactorial variance analysis of the effect of introduced rhizobacteria on the biomass and the contents of nutrient elements and heavy metals in the barley cv. Tselinnyi-5 plants cultivated in pots with the uncontaminated soil and soil contaminated with Pb and Cd at different concentrations

Introduced strain	Biomass, g/plant	Content of elements in plants					
		P, mg/g	K, mg/g	S, mg/g	Ca, mg/g	Cd, µg/g	Pb, µg/g
Uncontaminated soil							
Control (uninoculated plants)	2.55	3.3	19	2.3	6.9	0.26	0.84
<i>Az. lipoferum</i> 137	2.82*	3.3	17	2.0	6.0	0.38	0.70
<i>Ar. mysorens</i> 7	2.79*	3.5	19	2.3	7.0	0.31	0.96
<i>Ag. radiobacter</i> 10	2.75	3.5	20	2.7	7.0	0.32	0.98
<i>Flavobacterium</i> sp. L30	2.76	3.0	19	2.3	7.6	0.30	0.90
Soil with 100 mg Pb/kg							
Control (uninoculated plants)	2.54	2.4	16	2.1	6.0	0.42	1.31
<i>Az. lipoferum</i> 137	2.72	3.0	20**	2.3	5.3	0.32	1.42
<i>Ar. mysorens</i> 7	2.66	3.6**	20**	1.9	5.8	0.38	1.48
<i>Ag. radiobacter</i> 10	2.66	2.4	19*	1.8	6.0	0.43	1.29
<i>Flavobacterium</i> sp. L30	2.62	3.5**	20**	1.8	5.6	0.31	1.44
Soil with 500 mg Pb/kg							
Control (uninoculated plants)	2.45	2.6	16	1.6	5.1	0.32	2.99
<i>Az. lipoferum</i> 137	2.46	2.3	19**	2.3*	7.1**	0.32	2.97
<i>Ar. mysorens</i> 7	2.42	3.6**	22***	2.2	7.8**	0.33	2.93
<i>Ag. radiobacter</i> 10	2.57	3.8***	18	2.2	5.7	0.32	3.20*
<i>Flavobacterium</i> sp. L30	2.55	2.6	19*	2.2	4.9	0.31	3.28**
Soil with 30 mg Cd/kg							
Control (uninoculated plants)	1.25	3.0	25	2.2	6.9	1.87	0.94
<i>Az. lipoferum</i> 137	1.47*	2.5	25	3.3***	5.9	1.44	1.03
<i>Ar. mysorens</i> 7	1.26	3.4	26	3.3***	7.9	2.04	1.05
<i>Ag. radiobacter</i> 10	1.32	2.4	25	2.2	5.8	2.03	0.97
<i>Flavobacterium</i> sp. L30	1.38	2.3	26	2.4	6.8	2.20	1.02
Soil with 75 mg Cd/kg							
Control (uninoculated plants)	0.30	2.3	22	2.3	6.2	18.7	1.07
<i>Az. lipoferum</i> 137	0.52*	3.0*	23	1.9	6.5	15.2***	0.90
<i>Ar. mysorens</i> 7	0.47	2.7	21	2.7	5.8	17.1	1.05
<i>Ag. radiobacter</i> 10	0.52*	2.6	22	2.5	5.6	15.2***	0.95
<i>Flavobacterium</i> sp. L30	0.47	2.5	23	2.0	6.2	16.7*	0.93
Mean values for each of the experimental variants							
Uncontaminated soil	2.73	3.3	19	2.3	6.9	0.31	0.87
Soil with 100 mg Pb/kg	2.64	3.0	19	1.9*	5.7**	0.37	1.39***
Soil with 500 mg Pb/kg	2.49***	3.2	19	2.1	6.1*	0.32	3.07***
Soil with 30 mg Cd/kg	1.33***	2.7***	25***	2.7*	6.7	1.92***	1.00**
Soil with 75 mg Cd/kg	0.45***	2.6***	22***	2.3	6.1	16.6***	0.98*

Note: Here and in Tables 3–5, the asterisks mark significant differences between the control and inoculated barley plants according to Fisher's least significant difference test at $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***). In the case of mean values for each of the experimental variants, the asterisks mark significant differences between the barley plants cultivated in the control (uncontaminated) soil and in the soil contaminated with Pb and Cd.

Table 3. The bifactorial variance analysis of the effect of biopreparations on the grain yield and the contents of nutrient elements and heavy metals in grains of the barley cv. Abava cultivated on plots with the uncontaminated soil and soil contaminated with Pb (field experiment 1)

Biopreparation	Grain yield, g/m ²	Content of elements in grains				
		P, mg/g	K, mg/g	S, mg/g	Ca, mg/g	Pb, µg/g
Uncontaminated soil (14 ± 2 mg Pb/kg)						
Control (without inoculation)	425	3.3	6.2	1.5	0.76	0.39
Azorisin	413	3.4	5.9	1.5	0.74	0.28*
Mysorin	411	3.4	5.4	1.4	0.68	0.20**
Agrofil	368	3.8*	6.7	1.7*	0.77	0.33*
Flavobacterin	437	3.1	6.4	1.6	0.85	0.34
Soil with 100 mg Pb/kg						
Control (without inoculation)	411	3.3	5.6	1.3	0.84	0.39
Azorisin	427	3.0	5.5	1.5	0.76	0.29*
Mysorin	426	3.9*	7.3*	1.8**	0.87	0.35
Agrofil	398	3.9*	6.7*	1.5	0.77	0.39
Flavobacterin	444	3.2	6.1	1.7*	0.90	0.31*
Soil with 300 mg Pb/kg						
Control (without inoculation)	459	3.1	5.5	1.2	0.70	0.40
Azorisin	462	2.9	4.8	1.5*	0.71	0.30*
Mysorin	451	3.1	5.3	1.1	0.69	0.32*
Agrofil	416	3.7*	6.3	1.4	0.75	0.42
Flavobacterin	425	2.9	5.8	1.5*	0.78	0.37
Mean values for each of the experimental variants						
Uncontaminated soil	411	3.4	6.1	1.5	0.76	0.31
Soil with 30 mg Pb/kg	421	3.4	6.2	1.5	0.83	0.35
Soil with 200 mg Pb/kg	443	3.2	5.5	1.3	0.73	0.36

taminated with this heavy metal or not, whereas a beneficial effect of flavobacterin was observed only when the soil that was contaminated with 100 mg Pb/kg. In some experiments, the biopreparations enhanced the content of the nutrient elements P, K, and S, but not Ca, in the barley grain (Table 3).

In field experiment 2, the contamination of the plot soil with lead did not influence the grain yield, but diminished the content of P and S in the grain (Table 4). Treatment with azorisin and agrofil augmented the grain yield when the soil was contaminated with 300 mg Pb/kg and enhanced the content of P and K in the grain. In the case of the uncontaminated plot soil, treatment with azorisin increased the content of Ca and S in the barley grain. In addition, mysorin increased the P content of the grain. When the plot soil was contaminated with 100 mg Pb/kg, treatment with azorisin and flavobacterin augmented the content of Ca in the grain.

In field experiment 2, the contamination of the plot soil with cadmium led to its accumulation in the barley grain. In this case, the content of P in the grain decreased, whereas that of K increased (Table 5). Treatment with agrofil and flavobacterin increased the grain

yield when the soil was uncontaminated. Treatment with agrofil or azorisin diminished the accumulation of Cd in the grain when the plot soil was contaminated with either 5 or 15 mg Cd/kg. Mysorin enhanced the content of P and K, agrofil beneficially influenced the content of P, S, and K, and flavobacterin increased the content of S, K, and Ca in the grain.

When cultivated in vitro, the heavy metal-resistant rhizobacteria were found to be able to accumulate Cd ions (Fig. 2). The strains *Az. lipoferum* 137 and *Ag. radiobacter* 10, which reduced the content of Cd in barley plants to the greatest degree, exhibited the highest ability to accumulate Cd. It can be suggested that these strains prevent the uptake of this heavy metal by barley plants due to immobilization of Cd ions in the rhizosphere. On the other hand, the strain *Flavobacterium* sp. L30, which is relatively sensitive to Cd, nevertheless diminished the content of Cd in the barley plants. This favorable effect of *Flavobacterium* sp. L30 can presumably be accounted for by the ability of this strain to produce auxins in the barley rhizosphere, rather than by the direct immobilization of Cd ions. This suggestion is consistent with the earlier observa-

Table 4. The bifactorial variance analysis of the effect of biopreparations on the grain yield and the content of nutrient elements in grains of the barley cv. Abava cultivated on plots with the uncontaminated soil and soil contaminated with Pb (field experiment 2)

Biopreparation	Grain yield, g/m ²	Content of elements in grain				
		P, mg/g	K, mg/g	S, mg/g	Ca, mg/g	Pb, µg/g
Uncontaminated soil (19 ± 3 mg Pb/kg)						
Control (without inoculation)	339	5.9	5.3	2.2	0.76	0.22
Azorisin	392	6.8	6.7	3.0*	0.92*	0.19
Mysorin	320	7.3*	6.3	3.0*	1.01**	0.22
Agrofil	365	5.0	5.3	2.4	0.72	0.21
Flavobacterin	386	5.9	6.7	2.9	0.86	0.27
Soil with 100 mg Pb/kg						
Control (without inoculation)	326	4.4	5.3	1.8	0.75	0.26
Azorisin	350	6.1*	6.9*	2.2	0.99*	0.28
Mysorin	365	5.6	5.8	2.5	0.81	0.24
Agrofil	346	5.9*	6.7	2.5	0.88	0.24
Flavobacterin	349	6.9**	7.2*	2.5	0.94*	0.25
Soil with 300 mg Pb/kg						
Control (without inoculation)	322	4.1	4.9	2.2	0.80	0.28
Azorisin	392*	5.8*	6.0	2.5	0.88	0.30
Mysorin	306	5.8*	7.5**	2.2	0.94	0.23
Agrofil	420*	4.3	5.5	2.0	0.78	0.20
Flavobacterin	338	5.7*	6.7*	2.5	0.95	0.21
Mean values for each of the experimental variants						
Uncontaminated soil	360	6.2	6.1	2.7	0.85	0.25
Soil with 30 mg Pb/kg	347	5.8	6.4	2.3*	0.87	0.27
Soil with 200 mg Pb/kg	355	5.1**	6.1	2.3*	0.87	0.24

Table 5. The bifactorial variance analysis of the effect of biopreparations on the grain yield and the content of nutrient elements in grains of the barley cv. Abava cultivated on plots with the uncontaminated soil and soil contaminated with Cd (field experiment 2)

Biopreparation	Grain yield, g/m ²	Content of elements in grain				
		P, mg/g	K, mg/g	S, mg/g	Ca, mg/g	Pb, µg/g
Uncontaminated soil (0.08 ± 0.003 mg Cd/kg)						
Control (without inoculation)	310	4.4	6.3	1.8	0.73	0.16
Azorisin	360	5.1	5.7	2.0	0.70	0.15
Mysorin	309	5.5*	6.1	1.8	0.65	0.13
Agrofil	378*	6.3***	6.0	2.5*	0.80	0.14
Flavobacterin	373*	4.5	5.8	1.7	0.67	0.13
Soil with 5 mg Cd/kg						
Control (without inoculation)	363	4.5	5.6	1.9	0.70	0.37
Azorisin	339	4.0	5.6	1.6	0.68	0.27
Mysorin	344	5.5	7.3*	1.7	0.70	0.28
Agrofil	379	3.9	6.3	1.7	0.83*	0.20**
Flavobacterin	365	3.5	6.1	2.5*	0.71	0.26
Soil with 15 mg Cd/kg						
Control (without inoculation)	352	4.3	6.2	1.9	0.71	0.45
Azorisin	390	4.7	6.1	1.9	0.66	0.32*
Mysorin	366	5.7*	6.4	2.2	0.75	0.36
Agrofil	376	4.7	6.4	1.6	0.76	0.40
Flavobacterin	370	4.6	7.1*	2.5*	0.87*	0.39
Mean values for each of the experimental variants						
Uncontaminated soil	345	5.1	6.0	2.0	0.71	0.14
Soil with 5 mg Cd/kg	358	4.3**	6.2	1.9	0.72	0.28***
Soil with 15 mg Cd/kg	371	4.8	6.4*	2.0	0.75	0.38***

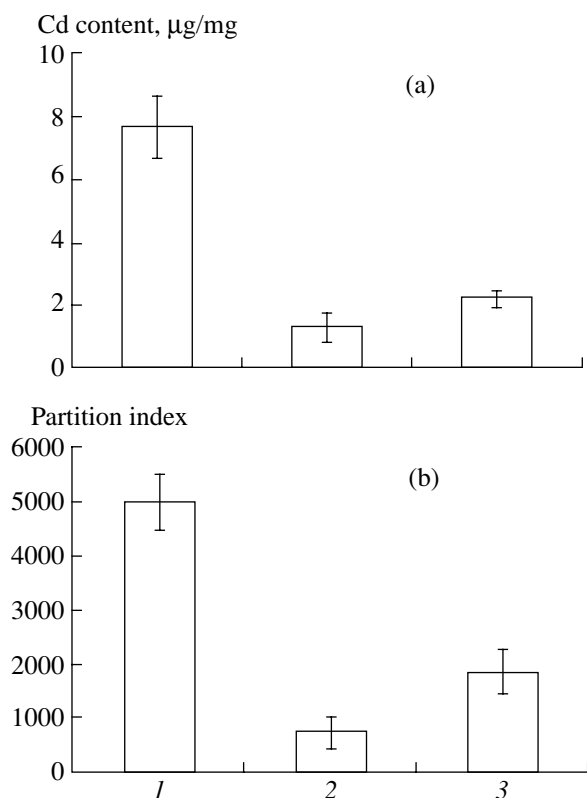


Fig. 2. The accumulation of cadmium by (1) *Az. lipoferum* 137, (2) *Ar. mysorens* 7, and (3) *Ag. radiobacter* 10 cells cultivated in liquid medium: (a) the content of cadmium in the dry bacterial biomass and (b) the partition index of cadmium between the bacterial biomass and the culture liquid. The error bars present standard deviations.

tion that *Flavobacterium* sp. L30 and the other rhizobacteria under study are able to actively synthesize auxins from tryptophan in the presence of toxic Cd concentrations and that cadmium even acts to enhance the synthesis of auxins [9].

The effect of the introduced rhizobacteria on the accumulation of Pb ions by the growing barley plants depended on the strain and the concentration of Pb ions in the soil. In the pot experiment with a high Pb contamination (500 mg Pb/kg soil), the inoculation of *Ag. radiobacter* 10 and *Flavobacterium* sp. L30 did not prevent the accumulation of Pb by the plants. In field experiment 1, when the barley plants were cultivated in the soil contaminated with 300 mg Pb/kg, these two strains also did not prevent the accumulation of Pb in the grain, although the strains *Az. lipoferum* 137 and *Ar. mysorens* 7 did reduce it.

Our earlier studies showed that the rhizobacteria and the respective biopreparations enhance the utilization of N and P from mineral fertilizers by barley plants cultivated in soil [15] and augment the content of P, K, Mg, Ca, Fe, Zn, and Mn in barley seedlings cultivated in hydroponic culture [9]. Similar data were obtained by

other researchers [16, 17]. The pot and field experiments described in the present paper confirm that the introduced rhizobacteria exert a beneficial effect on the uptake of various nutrient elements by barley plants cultivated in soil contaminated with Cd and Pb. It is known that the nutrient elements P, K, S, and Ca play an important part in the detoxification of heavy metals in plants and that heavy metals inhibit the assimilation of these nutrient elements by plants [9, 18]. The results obtained in this work are in good agreement with the earlier observations and show that the introduced rhizobacteria can mitigate the detrimental effect of heavy metals on the assimilation of nutrient elements by plants.

Thus, the rhizobacteria under study beneficially influenced the growth of the bacterized barley plants and their ability to assimilate nutrient elements from soil contaminated with Cd and Pb. The field experiments showed that the inoculation of barley seeds with the biopreparations based on these rhizobacteria can reduce and sometimes even prevent the accumulation of Pb and Cd in the grain. The introduction of the rhizobacteria into the barley rhizosphere mitigated the toxic effect of Cd and Pb on the plants cultivated in soil contaminated with these heavy metals.

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