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OCCURENCE OF METALRESISTANT MICROORGANISMS ON ISLANDS OF THE INTERNAL SHELF OF THE ANTARCTIC PENINSULA

Tashyrev A.B., Rokitko P.V., Matvieieva N.A., Tashyreva A.A., Romanovskaya V.A.

Zabolotny Institute of microbiology and virology of NAS of Ukraine, Ukraine, D 03680, Kiev, str., 154. E-mail: tach@i.com.ua

Abstract. Microorganisms, superresistant to toxic metals, have been revealed in a geographical zone of internal island shelf of Antarctic peninsula (30x60 km) on islands Darboux, Lippmann, Berthelot, Cruls, Barchans, Jalour, Uruguay, Scua, Three pigs, Winter, Grotto, Galindez, Pitermann, Booth, capes Perez, Rasmussen, Tuxen (coast of Antarctic peninsula), and also on distant from them island King-George. The highest number of metalresistant microorganisms is revealed on the medium containing 1000 ppm of Cr(VI) - up to $5{,}0x10^5$ cells/g of soil and on the medium containing 250 ppm of Ni^{2+} – $3{,}0\text{-}5{,}0x10^5$ cells/g of soil. Critical concentrations of toxic metals for the Antarctic microorganisms are determined: Hg^{2+} - 100 ppm, Cu^{2+} and Ni^{2+} - 500 ppm, Co^{2+} - 1000 ppm. For chromium this parameter is higher than 2000 ppm. For the analysis of results of frequency of metalresistant microorganisms occurrence used the factor representativeness (occurrence) C_{oc} suggested by us which allows to characterize in a complex geoobjects since connects two parameters: total of microorganisms and amount metalresistant microorganisms. Factor C_{oc} for nickelresistant and copperresistant microorganisms is practically identical on the investigated geoobjects. High C_{oc} are found out on cape Rasmussen and on island Winter, and the lowest - on island Lippmann. Thereby, we have shown for the first time, that resistance of microorganisms to ultrahigh concentration of toxic metals is widespread phenomenon in soils on an internal island shelf of the Western Antarctic Region. This conclusion is based on: a) superresistancies and polyresistance of the Antarctic microorganisms to toxic metals (Hg^{2+}, Cu^{2+}, Cr(VI), Ni^{2+}, Co^{2+}); b) high frequency of occurrence and high amount of metalresistant microorganisms in the Antarctic soil; c) occurrence of resistant to toxic metals microorganisms in all investigated soil samples in a zone of an internal island shelf of Antarctic peninsula in the extent 30x60 kms.

Key words: Antarctic Region, microorganisms, toxic metals, resistance, polyresistance.

1. Introduction

The phenomenon of resistance of the Antarctic microorganisms to toxic metals - one of the least studied aspects of the Antarctic microbiology. Only in a few publications the data concerning resistance to metals of water and ice bacteria are resulted (*De Souza et al.* 2006). Earlier we had been found out microorganisms resistant to toxic metals in the Antarctic samples selected on biogeographical polygon of island Galindez, (Tashyrev A.B. et al., 2007). At the same time, studying distribution resistant to toxic metals microorganisms in other geographical zones of Antarctic Region is of interest. In this connection, the purpose of investigation was studying of metalresistant microorganisms' distribution on the islands of an internal shelf and on the coast of Antarctic peninsula (30x60 km zone).

2. Methods and materials

Research objects were aerobic heterotrophic microorganisms isolated from native and frozen (-20°C) samples of soils. Samples were selected during 13-th Antarctic expedition on geographical objects (further in the text the term "geoobjects" is used) internal island shelf of Antarctic peninsula.

Preparation of solutions of toxic metals. Toxic metals brought in as solutions of salts $CuCI_2x2H_2O$, K_2CrO_4, $Hg(NO_3)_2$, $Ni(NO_3)_2x6H_2O$ и $CoCl_2x6H_2O$. Necessary concentration of toxic metals in a nutrient medium obtained by dilution of initial solutions: 20 g/l for Cu^{2+}, Co^{2+}, Ni^{2+}, Cr(VI)

and 4 g/l for Hg^{2+} (recalculation on a cation of metal). Nitrate of mercury obtained by dissolving 1 g metal mercury in 5,0 ml concentrated HNO_3. A solution evaporated on a water bath, and then $Hg(NO_3)_2$ dissolved in distilled water and lead up volume up to 1 liter. Solutions of metal ions sterilized during 20 min on boiling water bath, then cooled up to 30°C and brought in sterile conditions in a nutrient medium up to necessary final concentration.

Revealing of metalresistant microorganisms in samples was carried out on a liquid nutrient medium by standard methods. 0,1 g of sample placed in a tube which contained 10 ml NB medium (Nutrient Broth, HiMedia Laboratories Pvt. Ltd, the USA) with toxic metals and cultivated in stationary conditions at +20°C. Used such concentration of toxic metals in recalculation on a cation (ppm): Hg^{2+} - 100 and 200; Cu^{2+} - 500 and 1000; Ni^{2+} - 1000 and 2000; Co^{2+} - 1000 and 2000, Cr (VI) - 2000 and 5000. Presence of growth of microorganisms was determined on optical density of a culture liquid through with 3-14 day. The absence of growth of sensitive to metals strain *Escherichia coli* UCM B-906 served as the control of a toxicity of medium with metals.

Number of metalresistant and total amount of chemoorganotrophic microorganisms in researched samples determined by method of inoculation of consequent dilutions on agarized medium. To grinded in a porcelain mortar samples (1 g) added a physiological solution (10 ml) and mixed (1 hour). Then tenfold dilutions were inoculated on agarized medium NA (Nutrient Agar, firm HiMedia Laboratories Pvt. Ltd, the USA) which contained toxic metals (ppm): Hg^{2+} - 50 and 100, Cu^{2+} - 250 and 500, Ni^{2+} - 250 and 500, Co^{2+} - 500 and 1000, Cr (VI) - 1000 and 2000. The same dilutions were inoculated on an agarized media without metals for estimation of total amount of microorganisms. A cultivation carried out at +20°C. Colonies counted up after 7-10 days. Morphological types of colonies counted up in plates where their total number did not exceed 50. As the basic criteria for definition of morphological types of colonies on the base of such criteria: shape, diameter, consistence, pigmentation, synthesis of water soluble pigment, presence of extracellular slime, and presence of air and substrate mycelium. Single colonies selected and transferred on the same medium for their storage in laboratory conditions.

Occurrence frequency of metalresistant microorganisms determined by factor of representativeness C_{oc} suggested by us. This parameter calculated as a ratio of metalresistant microorganisms number in 1 g of sample and total amount of microorganisms selected on medium without metals: $C_{oc} = (N_{met} : N_{con}) 100\ \%$,

where C_{oc} is a representativeness factor; N_{met} – number of metalresistant microorganisms on a medium with metal; N_{con} - total amount of microorganisms on a medium without metals.

Polyresistance of microorganisms to metals determined by inoculation of representative strains on agarized medium, each of which contained on one toxic metal (ppm): Hg^{2+} – 50; Cu^{2+} – 250; Ni^{2+} – 250; Co^{2+} – 500; Cr(VI) – 1000.

3. Results and their discussion

Occurrence of metalresistant microorganisms on islands of an internal shelf of Antarctic peninsula. The investigated zone in the extent of 60 kms (from the North on the South) and 30 kms (from the West on the East) includes islands in the strait Penola and capes on the Antarctic peninsula. 18 biogeographical objects are investigated: islands Darboux, Galindez, Skua, Barchans, Uruguay, Jalour, Petermann, Berthelot, Cruls, Booth, Grotto, Three pigs, Winter, Lippmann, capes Rasmussen, Tuxen and Perez. Samples also were selected on island King-George which is considerably distant from the basic investigated zone.

For revealing metalresistant microorganisms screening soil samples on a liquid nutrient medium is carried out. It is established, that in a researched zone of Antarctic Region microorganisms superresistant to Cu^{2+} (500 ppm) and Cr(VI) (2000 ppm) (fig. 1) are widely ocurred. The attention that resistant microorganisms have been found out in 50-70 % of samples even at presence in medium 5000 ppm of chromium, and/or 1000 ppm of copper, and/or 1000 ppm of cobalt pay to itself. Such data testify to extremely high level of resistance of the Antarctic microorganisms to metal characterized by

high reductive-oxidative potential, that are very toxic because of oxidative properties, CrO_4^{2-} (E_o'=+555 мV for reaction of reduction CrO_4^{2-} to $Cr(OH)_3$) and also to Cu^{2+} - to metal which combines properties as the metal-substituent so the metal-oxidizer (E_o'=+440 мV for reaction of reduction Cu^{2+} to Cu_2O), that was discussed in previous work (Tashyrev A.B. et al., 2008). The most toxic for soil microorganisms appeared such metals as Hg^{2+} and Ni^{2+}. As a whole, microorganisms, superresistant to toxic metals, have been revealed on all investigated geoobjects of Antarctic Region. Therefore further we determined the quantitative characteristics of metalresistant microorganisms in some regions of Antarctic Region.

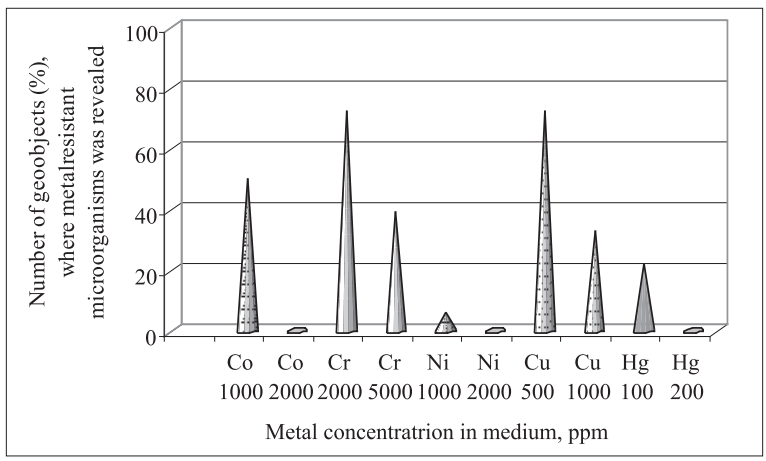

Fig. 1. Number of geoobjects (%) an internal island shelf of Antarctic peninsula in which microorganisms resistant to the specified concentration of toxic metals was revealed.

The quantitative account of metalresistant microorganisms has been carried out in soil of four geoobjects (islands Darboux, Lippmann, Winter and cape Rasmussen) by a method of inoculation of microorganisms from samples on agarized medium which contains the different toxic metals. The highest amount of metalresistant microorganisms is revealed on the medium with 1000 ppm of chromium - up to $5{,}0x10^5$ cells/g of soil, and on the medium with 250 ppm of nickel – $3{,}0\text{-}5{,}0x10^5$ cells/g of soil (tab. 1).

Table 1

Number of the metalresistant microorganisms revealed in soil samples

Metal concentration (ppm)	Number of metalresistant microorganisms in 1 g of soil			
	o. Darboux	c. Rasmussen	o. Winter	o. Lippmann
Co (500)	$1{,}3 \times 10^4$	$4{,}1 \times 10^4$	$2{,}1 \times 10^2$	$6{,}0 \times 10^2$
Co (1000)	$2{,}0 \times 10^3$	$5{,}0 \times 10^2$	nf	nf
Ni (250)	$3{,}5 \times 10^5$	$3{,}5 \times 10^5$	$2{,}5 \times 10^5$	$5{,}0 \times 10^5$
Ni (500)	nf	nf	$6{,}0 \times 10^2$	$5{,}0 \times 10^3$
Cr (1000)	$3{,}0 \times 10^4$	$6{,}0 \times 10^4$	$5{,}6 \times 10^5$	$4{,}2 \times 10^4$
Cr (2000)	$2{,}1 \times 10^3$	$6{,}0 \times 10^4$	$2{,}8 \times 10^5$	nf
Cu (250)	$2{,}7 \times 10^4$	$1{,}6 \times 10^4$	$1{,}5 \times 10^4$	nf
Cu (500)	$9{,}0 \times 10^2$	$9{,}0 \times 10^2$	$9{,}0 \times 10^2$	nf
Hg (50)	$1{,}0 \times 10^4$	$2{,}0 \times 10^4$	$2{,}5 \times 10^3$	$9{,}0 \times 10^2$
Hg (100)	nf	nf	nf	$2{,}0 \times 10^2$
Without metals	$1{,}0 \times 10^7$	$8{,}0 \times 10^6$	$9{,}0 \times 10^6$	$2{,}0 \times 10^7$

At higher concentration of these metals in the medium the amount of cells is less, though on separate geoobjects (for example, island Winter) the amount of resistant to chromium of microorganisms practically was not decreased. Thus, the Antarctic soil microorganisms showed significant resistance to high concentration Cr(VI).

At the same time, the amount of microorganisms on the medium with 50 ppm of mercury was in limits 1,0-3,0x10^2, and already at concentration of 100 ppm of mercury resistant microorganisms have been found out only on island Lippmann (tab. 1). For presentation in figure 2 the number of metalresistant microorganisms which is revealed in soil of island Darboux is resulted.

In these experiments critical concentration of toxic metals for the Antarctic microorganisms (in agarized medium) are determined: mercury - 100 ppm, copper - 500 ppm and cobalt - 1000 ppm (tab. 1). For chromium and nickel this parameter is not determined, as at used concentration of these metals (fig. 2) the significant amount of microorganisms (from 10^3 up to 10^5 cells/g of soil) is revealed. The established critical concentration of toxic metals for the Antarctic microorganisms are higher 1-2 order than for microflora of other regions that allows to count isolated microorganisms perspective for use in biotechnology of purification from metals of industrial waste waters.

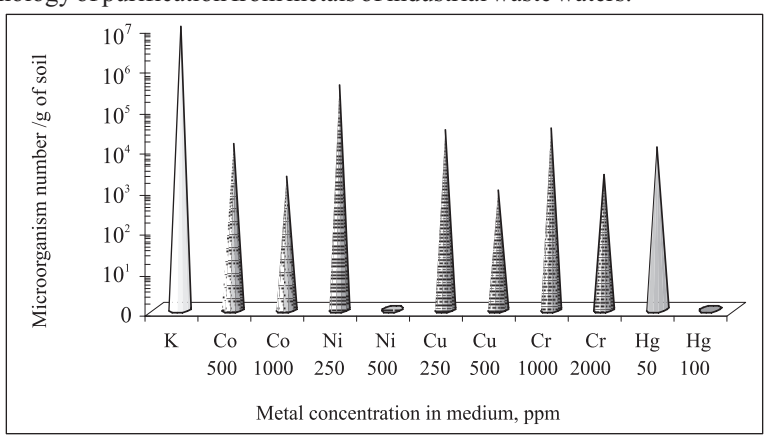

Fig. 2. Number of resistant to Co^{2+}, Ni^{2+}, Cu^{2+}, Cr(VI), Hg^{2+} microorganisms in 1 g of soil of island Darboux (K - number of microorganisms on the medium without metals).

Determination of occurrence frequency (factor of a representativeness) of metalresistant microorganisms. The total number of heterotrophic microorganisms (on the medium without metals) was in limits 0,8-2,0x10^7 cells/g of soil (tab. 1). Using this parameter and number of metalresistant Antarctic microorganisms (tab. 1) we calculated a factor of a representativeness (occurrence) of metalresistant microorganisms - C_{oc} (tab. 2).

Factor C_{oc} allows characterizing in a complex the phenomenon of metalresistance of microorganisms on the investigated geoobjects. It is shown, that the factor of C_{oc} nickelresistant microorganisms is practically identical on all investigated geoobjects. Similar factors are obtained and for copperresistant microorganisms, but with lower value (tab. 2). This law is distinctly visible in figure 3. The highest C_{oc} values are found out for samples of cape Rasmussen and on island Winter, and the lowest - on island Lippmann if to compare among themselves geoobjects (fig. 3).

As a whole, factor C_{oc} gives representation about relative occurrence frequency of metalresistant microorganisms and allows to characterize in a complex geoobjects since is a derivative of two characteristics: total number of microorganisms in a sample and amounts of metalresistant microorganisms among them.

Thus, obtained results of the microbiological analysis of soil samples have shown, that in the investigated biogeographical zone on all 18 geoobjects of an island shelf microorganisms, resistant to high concentration of toxic metals (Hg^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Cr(VI)) are found out.

Table 2

Factor of representativeness of metalresistant microorganisms on four investigated geoobjects of Antarctic Region

Metal concentration (ppm)	C_{oc} of metalresistant microorganisms , %			
	o. Darboux	c. Rasmussen	o. Winter	o. Lippmann
Co (500)	0,13	0,51	0,002	0,003
Co (1000)	0,02	0,006	-	-
Ni (250)	3,50	4,37	2,78	2,50
Ni (500)	-	-	0,007	0,025
Cr (1000)	0,30	0,75	6,22	0,21
Cr (2000)	0,021	0,75	3,11	-
Cu (250)	0,270	0,200	0,17	-
Cu (500)	0,009	0,01	0,01	-
Hg (50)	0,10	0,25	0,028	0,005
Hg (100)	-	-	-	0,001

The note: "-" - did not calculate, since on these geoobjects microorganisms, resistant to the specified metals have not been found out.

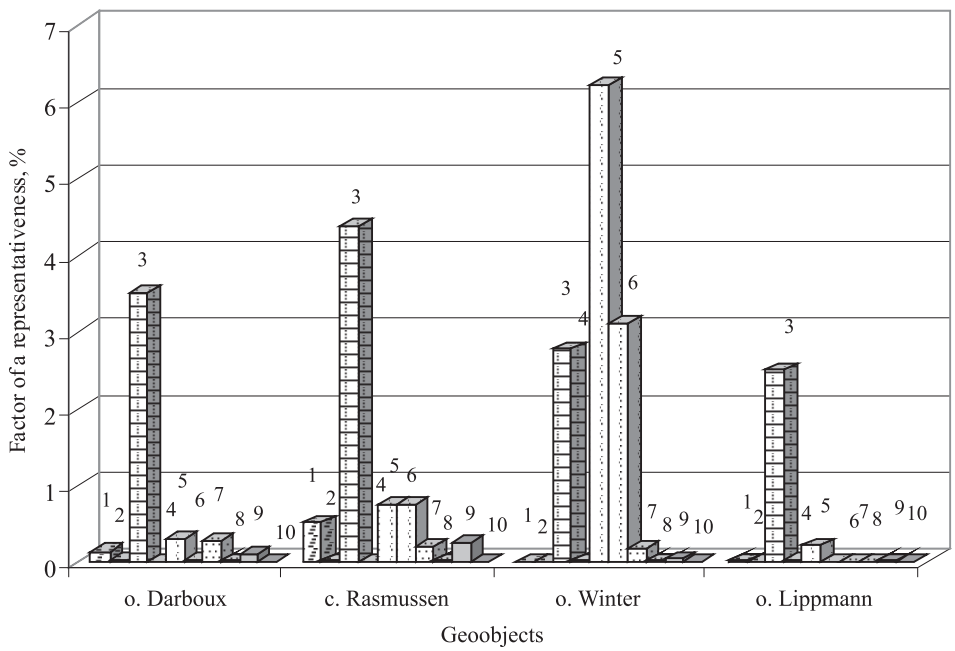

Fig. 3. Factors of representativeness *(C_{oc})* of the Antarctic metalresistant microorganisms. Figures above columns specify metal and its concentration in the medium (ppm): 1 – Co(500), 2 – Co(1000), 3 – Ni(250), 4 – Ni(500), 5 – Cr(1000), 6 – Cr(2000), 7 – Cu(250), 8 – Cu(500), 9 – Hg(50), 10 – Hg(100).

On the map-scheme (fig. 4) the geographical zone of an internal island shelf of Antarctic peninsula investigated by us (30x60 km), where the investigated geoobjects are designated, is resulted. As follows from the given scheme, on all investigated geoobjects of Antarctic Region (15 islands and 3 capes of Antarctic peninsula) have been revealed metalresistant microorganisms.

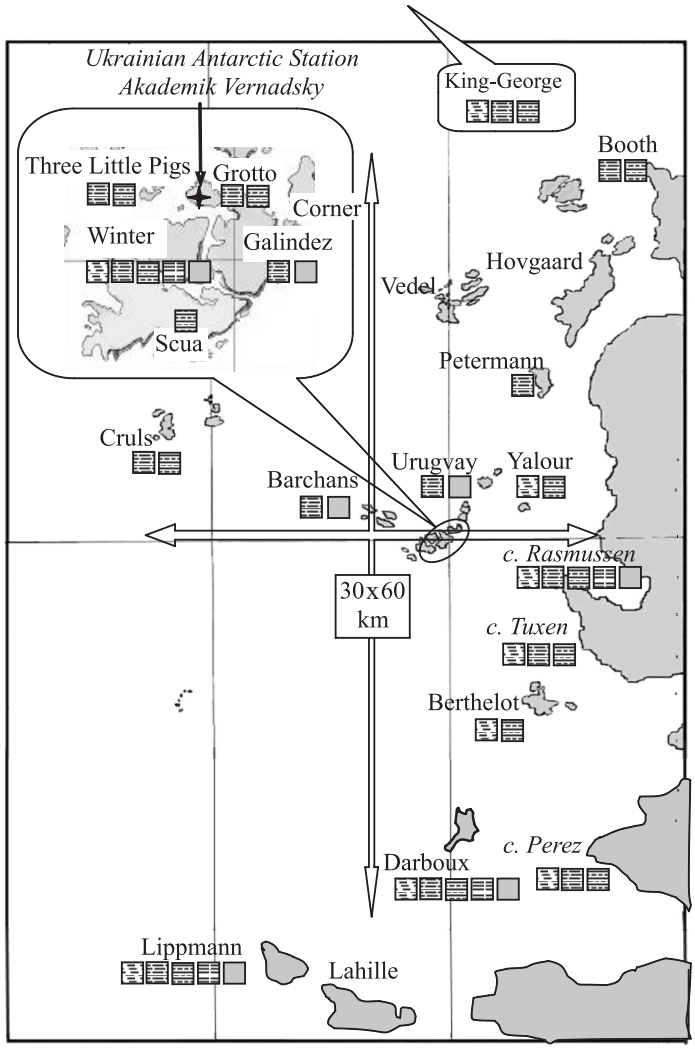

Fig. 4. The map - scheme of the investigated zone of an internal shelf of the Antarctic peninsula. The shaded squares under the place name mean, that in soil on this geoobject resistant microorganisms to the specified metal are revealed
- cobalt;
- cromium;
- copper;
- nikel;
- mercury.

Polyresistance of the Antarctic microorganisms. As a result of the carried out microbiological analysis of the soil samples selected on 18 geoobjects of an internal shelf of Antarctic peninsula, we developed a collection of metalresistant microorganisms (50 strains). At 21 strains (any way chosen from this collection) ability to grow has been investigated at the presence of different toxic metals (tab. 3). Polyresistance of microorganisms determined by inoculation strains on agarized medium which contained on one toxic metal in such concentration (ppm): Hg^{2+} – 50; Cu^{2+} – 250; Ni^{2+} – 250, Co^{2+} – 500 и Cr(VI) – 1000. It is shown, that 5 strains show resistance to all five metals, to four metals show resistance of 10 strains and to three metals - 5 strains (one strain has not shown polyresistance, that is was resistant only to one metal) (fig. 5).

Table 3

Polyresistancy to toxic metals of isolated Antarctic soil microorganisms

№ strain (concentration of metals at which has been selected a microorganism, ppm)	Resistance (R) and sensitivity (S) microorganisms at presence in the medium of metal (ppm)				
	Ni^{2+} (250)	Cu^{2+} (250)	Cr(IV) (1000)	Co^{2+} (500)	Hg^{2+} (50)
Resistance to 5 metals					
461($Ni^{R\ 500}$); 365($Cu^{R\ 250}$); 331($Cu^{R\ 250}$); 3216($Co^{R\ 500}$); 151($Hg^{R\ 50}$)	R	R	R	R	R
Resistance to 4 metals					
3215($Ni^{R\ 500}$)	R	R	R	R	S
121($Cu^{R\ 250}$); 141($Hg^{R\ 50}$); 391($Hg^{R\ 50}$); 381($Hg^{R\ 50}$); 411($Hg^{R\ 100}$)	R	R	R	S	R
341($Cu^{R\ 250}$); 3208($Co^{R\ 500}$); 3218($Hg^{R\ 100}$); 3217($Hg^{R\ 100}$)	R	R	S	R	R
Resistance to 3 metals					
306($Cr^{R\ 1000}$)	R	S	R	S	R
542($Ni^{R\ 250}$); 324($Cr^{R\ 1000}$); 432($Cr^{R\ 1000}$); 3223($Cr^{R\ 1000}$)	R	R	R	S	S

The note: R - presence of growth; S - absence of growth.

All researched microorganisms isolated on medium with mercury, appeared to be resistant as well to copper pays attention to it. On the other hand – all copperresistant microorganisms were resistant to mercury. These data allow suggests that, probably, the isolated strains have similar mechanisms of resistance to Cu^{2+} and Hg^{2+}.

It is necessary note that for strains of the microorganisms isolated from samples of polygon island Galindez (seasonal expeditions of 2001-2008), polyresistence to metals also is inherent (Tashyrev A.B. et al., 2007).

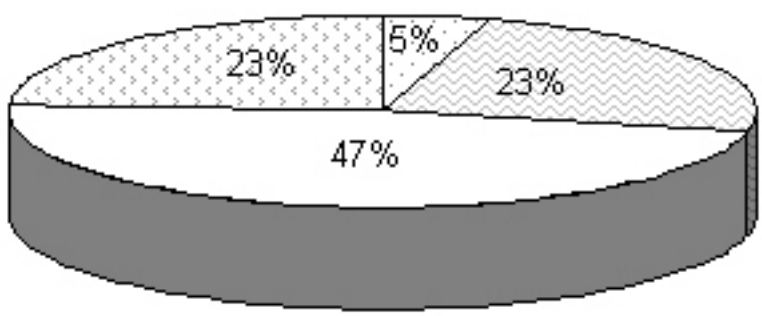

Fig. 5. Percent of strains characterized by polyresistance to metals: 47 % of the investigated microorganisms resistant to five metals, 23 % - to four and three metals (see tab. 3), 5 % - to one.

Thus, for the first time we have shown that resistance of microorganisms to ultrahigh concentration of toxic metals is widespread phenomenon in soil of an internal island shelf of the Western Antarctic Region. This conclusion is based on: a) superresistance and polyresistance of the Antarctic microorganisms to toxic metals (Hg^{2+}, Cu^{2+}, Cr(VI), Ni^{2+}, Co^{2+}); b) high frequency of occurrence and a significant amount of metalresistant microorganisms in the Antarctic soils; c) occurrence of resistant to toxic metals microorganisms in all investigated soil samples in a zone of an internal island shelf of Antarctic peninsula in the extent 30Ч60 kms.

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The information on authors

The address:
Ukraine, D 03680, Kiev, Zabolotnogo str., 154. Zabolotny Institute of microbiology and virology of NAS of Ukraine

Tashyrev A.B. - head of department of extremophilic microorganisms biology of Zabolotny Institute of microbiology and virology of NAS of Ukraine, Dr.Sci., senior scientific researcher, microbiologist, biotechnologist. Ph. 8 (044) 526-32-97. E-mail: tach@i.com.ua

Rokitko P.V. - senior scientific researcher of department of extremophilic microorganisms biology of Zabolotny Institute of microbiology and virology of NAS of Ukraine, Ph.D., microbiologist, ecologist. Ph. 8 (044) 526-32-97.

Matveeva N.A. - leading engineer of department of extremophilic microorganisms biology of Zabolotny Institute of microbiology and virology of NAS of Ukraine, Ph.D., microbiologist, biotechnologist.

Tashyreva A.A. - leading engineer of department of extremophilic microorganisms biology of Zabolotny Institute of microbiology and virology of NAS of Ukraine, microbiologist.

Romanovskaya V.A. - leading scientific researcher of department of extremophilic microorganisms biology of Zabolotny Institute of microbiology and virology of NAS of Ukraine, Dr.Sci., professor, microbiologist, ecologist.