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**Ivanitsa V. A.,** Dr. of Biol. sciences, Prof., Head of Dept.,
**Buchtiyarov A. Ye.,** Professor's assistant
Odessa State University, Department of Microbiology and Virology,
Dvoryanskaya St. 2, Odessa, 65026, Ukraine

# RESISTANCE LEVEL TO HEAVY METALS OF MICROBIAL COENOSES OF THE BLACK SEA SHOREFACE [1]

The minimal concentrations of $Hg^{2+}$, $Pb^{2+}$, $Cd^{2+}$ ions that inhibit the growth of heterotrophic marine bacteria on dense nutritious medium has been determined. There has been shown that the quantity of bacteria that are resistant to the investigated metals is higher an order in regions with high technogenic load (the Datcha Kovalevskogo and the Oil terminal of the Odessa port).

**Key words:** marine bacteria, ions of $Hg^{2+}$, $Pb^{2+}$, $Cd^{2+}$, resistance.

Chemical pollution by heavy metals has become one of the actual problems of anthropogenic influence on marine environment. Their characteristics are high bioaccumulation ability, formation of new complex compounds, toxicity in small concentration and gradual accumulation in the environment that results in ecosystem degradation.

Mercury, lead and cadmium, which enter at direct pollution, through atmosphere and with continental drain, are the most dangerous for water biocoenoses [2]. It is supposed that toxicants by rendering pressure on the genetic apparatus form conditions for microorganisms' technogenic microevolution in direction of increasing their aggressiveness [1]. Therefore, for understanding of the processes that determine assimilation capacity of the system it is interesting to study an ability of microbial coenoses with various levels of toxic agents' action to react on stressing concentrations of prior toxicants.

The purpose of the present work has been to determine the levels of resistance to $Hg^{2+}$, $Pb^{2+}$ and $Cd^{2+}$ ions for heterotrophic marine bacteria of microbial coenoses in Odessa region shoreface.

## Materials and methods

The samples of marine water have been selected at the Delfin Beach, at the mooring № 138 in the region of Datcha Kovalevskogo, at the Central Oil terminal of Odessa Port at the mooring № 1 (The Oil terminal), and also from the Dniester river in region of the settlement Mayaki. The selection has been carried out from superficial water horizon (0—50 cm) in July 1999.

The quantity of heterotrophic bacteria has been determined by inoculation method on Горбенко solid culture medium [4]. 9 ml of the culture medium has been brought into a petri dish and 1 ml of concentrated solution of the toxicant in the experiment (or sterile marine water in the control) has been added, so that each of 10 ml of the culture medium would contain the needed concentration. The range of heavy metal concentrations has been selected on the basis of preliminary research. Salts of heavy metals $HgCl_2$

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($Hg^{2+}$), Pb ($NO_3$)$_2$ ($Pb^{2+}$) and $CdCl_2$ ($Cd^{2+}$) have been used as toxicants in the following dilutions: $Hg^{2+}$ (0,005; 0,01; 0,05; 0,1; 0,5 mmol · liter$^{-1}$), $Pb^{2+}$ (0,1; 0,5; 1; 1,5; 2 mmol × × liter$^{-1}$) and $Cd^{2+}$ (0,05; 0,1; 0,5; 1; 1,5 mmol · liter$^{-1}$).

Cultivation of bacteria has been carried out at temperature 22 °C during 48 hours. The minimal concentration of the toxicant, at which the growth of microorganisms has not been observed, is assumed as minimal inhibitory concentration (MIC) of the toxicant. The contents of toxicants in water samples have been determined with the help of atom-adsorption determination according to the method [3].

## Results and discussion

The study of contents of the toxic metals ($Hg^{2+}$, $Pb^{2+}$ and $Cd^{2+}$) has shown, that they have been present in natural water of the investigated region in the minimal concentrations that do not exceed levels of Maximum Concentration Limit (MCL) [5] (Table 1). Mercury has been found in trace quantities, and the maximal content of lead and cadmium has not exceed $1,5 \cdot 10^{-5}$ mmol · liter$^{-1}$ and $2,8 \cdot 10^{-6}$ mmol · liter$^{-1}$, correspondingly. The received results confirm the opinion that at this moment there is normalization of the ecological situation concerning the contents of heavy metals in the North-Western part of the Black Sea and the Dniester river [6].

Table 1

**Contents of heavy metal ions (mmol·liter$^{-1}$) in water of investigated regions**

| Ion | MCL* | The Oil terminal | The Delfin Beach | Datcha Kovalevskogo | Dniester |
|---|---|---|---|---|---|
| $Pb^{2+}$ | $1,5 \cdot 10^{-4}$ | $1,5 \cdot 10^{-5}$ | $6,3 \cdot 10^{-6}$ | $1,3 \cdot 10^{-5}$ | $1,4 \cdot 10^{-5}$ |
| $Cd^{2+}$ | $8,9 \cdot 10^{-6}$ | $2,6 \cdot 10^{-6}$ | $1,6 \cdot 10^{-6}$ | $2,8 \cdot 10^{-6}$ | $2,2 \cdot 10^{-6}$ |
| $Hg^{2+}$ | $2,5 \cdot 10^{-6}$ | trace | trace | trace | trace |

Note: * — Maximum Concentration Limit.

Inoculation of the selected water samples on cultural medium, containing the given concentration of one or another metal, enables to determine what share of microorganisms from the total number contained in the sample is resistant to this concentration of the metal and is able to grow in its presence.

The analysis of the data that is presented in Tables 2, 3 and 4 testifies that in the investigated water microbial coenoses there are representatives that are resistant to high concentration of toxic metals.

So, the complete absence of bacterial growth has been observed at inoculation of water samples from the Delfin Beach and the Dniester on the medium with lead in concentration of 1.0 mmol · liter$^{-1}$ and from region of the Datcha Kovalevskogo and the Oil terminal in concentration of 1,5 mmol · liter$^{-1}$ (Table 2). That is, these concentrations of metal ions should be considered as MIC for all bacteria contained in water samples without exception. Shares of bacteria that have grown on the medium with $Pb^{2+}$ in concentration below MIC (0,5 mmol · liter$^{-1}$) has been 29,2% for bacteria from the Dniester, 15,5% for bacteria from water of the Delfin Beach, and on the medium with $Pb^{2+}$ in concentration 0,1 mmol · liter$^{-1}$ it has been 51,7% and 78,0% corresponding-

Table 2

**Quantity of bacteria (CFU[a] · ml$^{-1}$) during growth on media with $Pb^{2+}$**

| Concentration of $Pb^{2+}$ (mmol · liter$^{-1}$) | The Oil terminal | | The Delfin Beach | | Datcha Kovalevskogo | | Dniester | |
|---|---|---|---|---|---|---|---|---|
| | Abs Num[b] | Share (%) | Abs Num[b] | Share (%) | Abs Num[b] | Share (%) | Abs Num[b] | Share (%) |
| Control | 9540±319 | 100,0 | 2710±373 | 100,0 | 3693±393 | 100,0 | 2770±287 | 100,0 |
| 0,1 | 9165±215 | 96,1 | 1400±190 | 51,7 | 3152±255 | 85,4 | 2160±285 | 78,0 |
| 0,5 | 4075±200 | 42,7 | 420±48 | 15,5 | 1270±210 | 34,4 | 810±62 | 29,2 |
| 1,0 | 2120±195 | 22,2 | 0 | 0,0 | 100±16 | 2,7 | 0 | 0,0 |
| 1,5 | 0[c] | 0,0 | 0 | 0,0 | 0 | 0,0 | 0 | 0,0 |

Note: [a] — Abbreviations: Colony Formation Unit; [b] — Absolute Number. Results are reported as the means ± standard deviations of three determinations; [c] — MIC.

Table 3

**Quantity of bacteria (CFU · ml$^{-1}$) during growth on media with $Cd^{2+}$**

| Concentration of $Cd^{2+}$ (mmol · liter$^{-1}$) | The Oil terminal | | The Delfin Beach | | Datcha Kovalevskogo | | Dniester | |
|---|---|---|---|---|---|---|---|---|
| | Abs Num | Share (%) | Abs Num | Share (%) | Abs Num | Share (%) | Abs Num | Share (%) |
| Control | 9540±319 | 100,0 | 2710±373 | 100,0 | 3693±393 | 100,0 | 2770±287 | 100,0 |
| 0,05 | 9200±353 | 96,4 | 1230±251 | 45,4 | 3150±299 | 85,3 | 2510±120 | 90,6 |
| 0,10 | 4890±378 | 51,3 | 690±102 | 25,5 | 1810±163 | 49,0 | 1790±262 | 64,6 |
| 0,50 | 1010±117 | 10,6 | 20±2 | 0,7 | 470±83 | 12,7 | 0 | 0,0 |
| 1,00 | 0 | 0,0 | 0 | 0,0 | 0 | 0,0 | 0 | 0,0 |

Table 4

**Quantity of bacteria (CFU · ml$^{-1}$) during growth on media with $Hg^{2+}$**

| Concentration of $Hg^{2+}$ (mmol · liter$^{-1}$) | The Oil terminal | | The Delfin Beach | | Datcha Kovalevskogo | | Dniester | |
|---|---|---|---|---|---|---|---|---|
| | Abs Num | Share (%) | Abs Num | Share (%) | Abs Num | Share (%) | Abs Num | Share (%) |
| Control | 9540±319 | 100,0 | 2710±373 | 100,0 | 3693±393 | 100,0 | 2770±287 | 100,0 |
| 0,005 | 9490±419 | 99,5 | 1680±177 | 62,0 | 2550±153 | 69,0 | 2210±207 | 79,9 |
| 0,010 | 8720±329 | 91,4 | 10±3 | 0,4 | 400±26 | 10,8 | 930±39 | 33,6 |
| 0,050 | 6210±236 | 65,1 | 0 | 0,0 | 90±9 | 2,4 | 0 | 0,0 |
| 0,100 | 0 | 0,0 | 0 | 0,0 | 0 | 0,0 | 0 | 0,0 |

ly. Resistance of microbiota is significantly higher in the region of the Datcha Kovalevskogo and the Oil terminal and higher MIC levels testify about that.

The shares of resistant representatives in microbial cenosis are especially great in the Oil terminal. 22,2% of microbial representatives from the Oil terminal have displayed an ability to grow at concentration of $Pb^{2+}$ 1.0 mmol · liter$^{-1}$, which is MIC for

microbiota in the region of the Delfin Beach and the Dniester. As it is shown in Table 3, the microbiota from water of the Dniester river has appeared to be the least resistant to $Cd^{2+}$ ions. MIC of $Cd^{2+}$ has appeared to be 0,5 mmol · liter$^{-1}$ for it. Microbial coenoses of the Delfin Beach, of the Datcha Kovalevskogo and of the Oil terminal have shown two times higher resistance.

MICs of $Cd^{2+}$ for them has been at the level of 1.0 mmol· liter$^{-1}$. It is interesting to note that resistance to such $Cd^{2+}$ concentration has been shown just by 0,7% of microbial cenosis from the Delfin Beach water, while in region of the Datcha Kovalevskogo and in the Oil terminal it has been shown by 12,7% and 10,6% correspondingly.

As it follows from Table 4, MIC of mercury for microbial communities in the region of the Delfin Beach and the Dniester river have been 0,05 mmol · liter$^{-1}$, and for the Datcha Kovalevskogo and the Oil terminal MICs of $Hg^{2+}$ as well as of $Pb^{2+}$ have been 0,1 mmol · liter$^{-1}$, which is two times higher. Thus, at concentration of $Hg^{2+}$ below minimal inhibiting concentration of 0.01 mmol · liter$^{-1}$, the share of the resistant representatives from water of the Delfin Beach and the Dniester river has been 0,4% and 33,6% correspondingly. The highest contents of resistant bacteria has been revealed in the Oil terminal microbial cenosis. At equal values of MIC of $Hg^{2+}$ (0,1 mmol · liter$^{-1}$) for microbial population in region of the Datcha Kovalevskogo and of the Oil terminal, significantly larger share of resistant microorganisms has been revealed for the Oil terminal bacteria. The quantity of resistant microorganisms, that have grown on medium with $Hg^{2+}$ concentration following MIC, has been 2,4% for the Datcha Kovalevskogo, while it has been 65,1% for the Oil terminal.

While analyzing the presented results, it is possible to ascertain that microbial coenoses of the investigated regions show resistance to ions of lead, cadmium and mercury in a similar way. The level of microbiota's resistance to all of the three metals grows in the following sequence of regions: the Delfin Beach, the Dniester river, Datcha Kovalevskogo and the Oil terminal.

Thus, regardless of the fact that in water of the investigated regions the contents of $Pb^{2+}$, $Cd^{2+}$, $Hg^{2+}$ are insignificant and even below than Maximum Concentration Limit, microbial coenoses show their ability to grow at concentrations of the given metals, which are 5–6 orders higher. It can be a manifestation of genetically determined natural mechanisms of bacterial resistance to heavy metals.

At the same time, in our opinion, the distinctions in resistance to $Pb^{2+}$, $Cd^{2+}$ and $Hg^{2+}$, which have been shown by microbial coenoses of the investigated regions, are very important. The greatest resistance to heavy metals has been revealed for the microbial population of the shoreface of the Oil terminal and the Datcha Kovalevskogo, that is quite in accordance to the fact that microbial coenoses in these regions are subject to significant anthropogenic pollution. Waste water of biological treatment station "Yuzhnaya" is dumped in region of the Datcha Kovalevskogo, and there is an exit of the municipal collector and technogenic influence of the Port in the Oil terminal.

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**Іваниця В. О., Бухтіяров А. Є.**
Одеський державний університет, каф. мікробіології та вірусології,
вул. Дворянська, 2, Одеса, 65026, Україна

## РІВЕНЬ РЕЗИСТЕНТНОСТІ ДО ВАЖКИХ МЕТАЛІВ МІКРОБНИХ ЦЕНОЗІВ ПРИБЕРЕЖНИХ ВОД ЧОРНОГО МОРЯ

**Резюме**

Визначені мінімальні концентрації іонів $Hg^{2+}$, $Pb^{2+}$, $Cd^{2+}$, що інгібують ріст гетеротрофних морських бактерій на щільному живильному середовищі. Виявлено, що кількість резистентних до досліджених металів бактерій на порядок вище у районах з високим техногенним навантаженням (район Дача Ковалевського і Нафтогавані Одеського порту).

**Ключові слова:** морські бактерії, іони $Hg^{2+}$, $Pb^{2+}$, $Cd^{2+}$, резистентність.

**Иваница В. А., Бухтияров А. Е.**
Одесский государственный университет, каф. микробиологии и вирусологии,
ул. Дворянская, 2, Одесса, 65026, Украина

## УРОВЕНЬ РЕЗИСТЕНТНОСТИ К ТЯЖЕЛЫМ МЕТАЛЛАМ МИКРОБНЫХ ЦЕНОЗОВ ПРИБРЕЖНЫХ ВОД ЧЕРНОГО МОРЯ

**Резюме**

Определены минимальные концентрации ионов $Hg^{2+}$, $Pb^{2+}$, $Cd^{2+}$, ингибирующие рост гетеротрофных морских бактерий на плотной питательной среде. Показано, что количество резистентных к исследуемым металлам бактерий на порядок выше в районах с высокой техногенной нагрузкой (Дача Ковалевского и Нефтегавань Одесского порта).

**Ключевые слова:** морские бактерии, ионы $Hg^{2+}$, $Pb^{2+}$, $Cd^{2+}$, резистентность.