ZOOLOGIE

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EVALUATION OF THE ACTION OF SOME COORDINATION COMPOUNDS ON INFUSORIA *PARAMECIUM CAUDATUM* (Ehrenberg, 1833) VIABILITY

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Rezumat

Articolul oferă noi perspective asupra activității biologice a sărurilor de sodiu și litiu ale complexului binuclear $[Mo_2O_4(EDTA)]^{2-}$ – un model molecular sintetic binecunoscut al centrului activ al molibdoenzimelor. Experiențele clasice cu ciliatul standard *Paramecium caudatum* (Ehrenberg, 1833) demonstrează un efect stimulator neașteptat asupra viabilității acestora, crescând semnificativ rata lor de reproducere la concentrații micromolare ale sării de litiu în mediul nutritiv.

Cuvinte cheie: Paramecium caudatum, biotestare, molibden, compus coordinativ, substanțe bioactive, mediu.

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Introduction

Biotesting, as a research method, is used in different fields of science, for example, in ecology – for water and soil analysis, in medicine – for studying the properties of internal environments of higher organisms, in agriculture – for rapid testing of forage toxicity, in chemistry – for the initial assessment of the property of newly synthesized substances [5, 7, 10]. Biotesting is an evaluation of the properties of the studied substance in dependence of its effects on the experimental organism under laboratory conditions [9].

One biological property for many compounds that earned good reputation so far seems to be the *in vivo* cytotoxic effect on the protozoa population, as shown hereafter. As objects of study served infusoria of the genus *Paramecium (*sp. *Paramecium caudatum).* The advantage of the biotesting with *Paramecium caudatum* as test-object consists in its human controlled approach, rapid investigation, besides, it needs significantly lower volumes of the substances to be tested.

Molybdenum (Mo) is an essential microelement since it is found at the heart of molybdoenzymes, so much vital for the living organisms. Since this metal plays an active

and important role in the metabolism of the majority of Earth's life forms, developing new Mo-based compounds thus appears as a good strategy to achieve promising drugs or biomimetic molecules for biochemical and biomedical research [1].

Out of all functions (test-reactions) of single-celled organisms, which occur under the influence of certain factors (stimulators, retardants and inhibitors), the most substantial are the following: the viability of the population, negative or positive fluctuation of population dynamics, and the reproduction rate [4].

Toxicity testing represents an important phase in the development of medicinal products and is a prerequisite before starting their use in preclinical and clinical trials. Since the fundamental principle of toxicity studies is the protection of animals, including those used in experimental studies, it is now recommended that in all possible cases, to conduct experiments *in vivo* on single-cell organisms, avoiding the use of laboratory animals.

The main goal was to study the compounds with biologically active properties, as well as their influence on the population dynamics, the reproduction rate and the viability of the tested organisms.

Materials and methods

The methodology applied in this scientific research was carried out on the basis of the concepts reported in several papers [2, 3, 6, 8].

Unicellular organisms *Paramecium caudatum* were used as test objects to detect the eventual toxicity of applied active substances. Thus, the action of the complexes $Li_2[Mo_2O_4(EDTA)]\cdot 5H_2O$ and $Na_2[Mo_2O_4(EDTA)]\cdot 5H_2O$ (herein denoted MoLi and MoNa) was studied by monitoring the viability of test-organisms. The biologically active substances were synthesized in the Lavoisier Institute of Versailles (France).

The preparations were tested at concentrations of 100, 10, 1 and 0.1 μM compared to the control group. Counting of the total number of cells in the environment containing ciliates was done by using a binocular microscope. The mean value of the number of paramecia was calculated after 24, 48 and 24-144 hours. The incubation temperature was of 28^0 C.

In order to assess the toxic effects in cell cultures, LC_{50} – the concentration of compounds, which reduce the cell viability by 50% – was determined. The obtained data were statistically processed using the program Excel and the median lethal concentration (LC_{50}) values were calculated by using GraphPad.

Results and discussions

The natural sensibility of organisms for tested substances varies depending on the administered doses. According to the method [2], *Paramecium caudatum* showed a high responsiveness to the action of the tested compounds.

To run the assay, live *P. caudatum* cells (3×10^3 cells/mL) were exposed to various concentrations of the tested compounds for 24 and 48 hours.

After 24 h from the beginning of investigations, the viability of paramecia in the experimental group, to which the complex MoLi was administered, was of 87.7% (100 μM), 91.0% (10 μM), 90.5% (1 μM) and 90.8% (0.1 μM) compared to the control group. After 48 h the viability of organisms was of 76.3% (100 μM), 86.3% (10 μM), 91.8% (1 μM) and 99.3% (0.1 μM). Further, the viability increased at concentrations of 1-0.1 μM, with an insignificant decrease at concentrations of 10-100 μM (tab.1; fig.1).

Table 1: Viability (%) of ciliate *P. caudatum* under the action of biologically active substances MoLi and MoNa in various concentrations after 24 and 48 h of incubation

Compound	Conc. (μM)	24 h			48 h		
		Viability (%)	SD	LC_{50}	Viability (%)	SD	LC_{50}
MoLi	100	87.7	8.3	≥100	76.3	10.5	≥100
	10	91.0	6.6		86.3	3.5	
	1	90.5	14.3		91.8	15.0	
	0.1	90.8	5.2		99.3	16.8	
MoNa	100	52.4	1.1	≥100	77.2	6.0	≥100
	10	76.9	7.9		104.6	1.9	
	1	78.3	12.4		106.0	2.8	
	0.1	73.0	19.0		103.3	23.7	

When administering the preparation MoNa, the viability index was equal to 52.4% (100 μM), 76.9% (10 μM), 78.3% (1 μM) and 73.0% (0.1 μM) after 24 h. After 48 h from administering the complex, the viability increased insignificantly, with values of 77.2% (100 μM), 104.6% (10 μM), 106.0% (1 μM) and 103.3% (0.1 μM) compared to the control group (fig.2).

LC_{50}≥100 in the case of MoLi, as well as MoNa, but the trend of viability indicated that the used compounds have a stimulatory effect after 48 hours (tab.1).

The obtained scientific results have revealed the need for further examinations of the substances MoLi and MoNa, according to hydrobiological standard methods [3, 6, 8]. To run the assay, live *P. caudatum* cells (1 cell/ 1 mL) were exposed to concentrations of 100, 10, 1 and 0.1 μM compared to the control group. The incubation time was of 24-144 hours, at 28 ^{0}C.

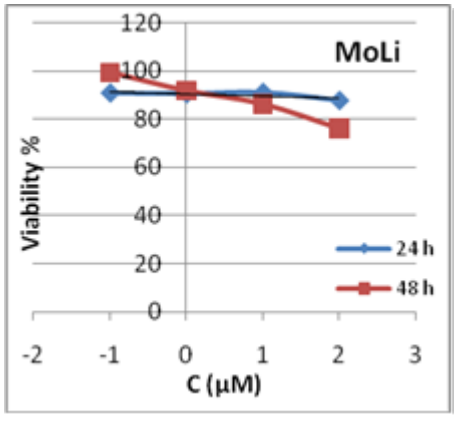

Figure 1: Viability of ciliate *P. caudatum* at the action of substance MoLi, in concentrations of 100, 10, 1 and 0.1 μM, for 24 and 48 h.

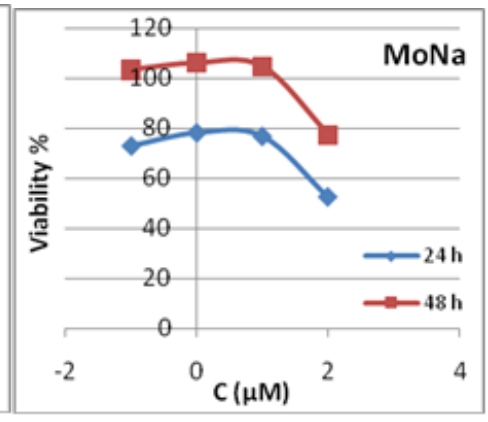

Figure 2: Viability of ciliate *P. caudatum* at the action of substance MoNa, in concentrations of 100, 10, 1 and 0.1 μM, for 24 and 48 h.

During the first 48 h, there was an increase in the viability of the tested organisms. The mean value, after 72 h of addition of MoLi compound, was of 604.3% (100 μM), 460.9% (10 μM), 430.4 % (1 μM) and 337.0 % (0.1 μM) compared to the control group. After 96 h since the beginning of experiment, the index, on average, increased significantly to 1063.9% (100 μM), to 2543.1% (10 uM), up to 2782.4% (1 μM) and up to 2537.3% (0.1 μM) compared to the control. After 120 and, respectively, 144 h of experiment, the index decreased at all used concentrations (tab. 2).

Table 2: Modification of viability (%) of ciliate *P. caudatum* under the action of biologically active compound MoLi during incubation

Incubation period, h	Viability, %			
	MoLi (0.1 μM)	MoLi (1 μM)	MoLi (10 μM)	MoLi (100 μM)
24	145.9	170.3	127.0	148.6
48	215.7	174.5	221.6	213.7
72	337.0	430.4	460.9	604.3
96	2537.3	2782.4	2543.1	1603.9
120	480.1	409.7	446.8	354.7
144	433.6	415.6	448.9	258.2

Thus, during the experiment the viability of cells have permanently increased, reaching its peak after 96 h of incubation, when, for example, in the experimental group with addition of 1 μM MoLi the number of cells was about 8 times higher than in the control group (fig.3).

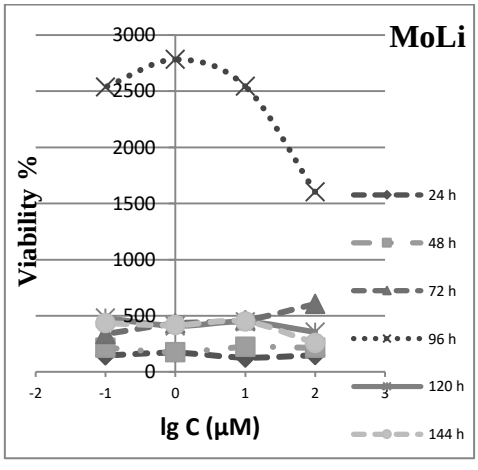

Figure 3: Viability of ciliate *P. caudatum* at the action of MoLi, in concentrations of 100, 10, 1 and 0.1 μM, for 144 h

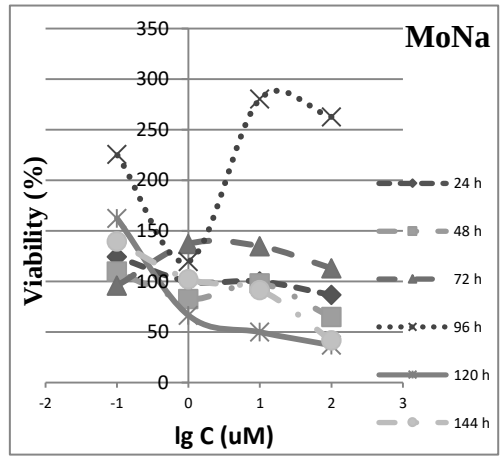

Figure 4: Viability of ciliate *P. caudatum* at the action of MoNa, in concentrations of 100, 10, 1 and 0.1 μM, for 144 h

MoNa registered the same tendency as MoLi of increasing the viability of individuals after 72 h of incubation, reaching its peak after 96 h. However, the values of viability in the case of MoNa were much lower (tab. 3).

Table 3: Modification of viability (%) of ciliate *P. caudatum* under the action of biologically active compound MoNa during incubation

Incubation period, h	Viability, %			
	MoNa (0.1 μM)	MoNa (1 μM)	MoNa (10 μM)	MoNa (100 μM)
24	124.3	100.0	100.0	86.5
48	109.8	82.4	98.0	64.7
72	95.7	137.0	134.8	113.0
96	225.5	119.6	280.4	262.7
120	162.4	66.4	49.8	36.8
144	139.4	102.2	91.5	41.6

For the paramecia in the experimental group, after 96 h of incubation, the mean value of viability increased up to 262.7% (100 μM), 280.4% (10 μM), 119.6% (1 μM) and 225.5% (0.1 μM). A significant decrease of paramecia viability was registered after 120 h of addition of MoNa – 36.8% (100 μM), 49.8% (10 μM), 66.4% (1 μM) and 146.5% (0.1 μM) in comparison with the control group (fig.4).

The most essential changes of the paramecium viability were recorded in the case of addition of MoLi solution. According to the tension series of metals, Li has a higher reactivity (property to interact) than Na. This can be the cause of discrepancy between the obtained results for MoLi and MoNa. The experimental results proved that such compounds as MoNa and MoLi are not toxic to tested *P. caudatum* infusoria, moreover they increase the productivity.

The test compound MoLi has been recommended as a supplement for *Apis mellifera* bee families, with the aim of increasing their productivity.

Conclusions

The experimental works revealed that coordinating compound MoLi has a more pronounced influence on the viability of *P. caudatum* in comparison with MoNa. During the first 96 h of paramecia incubation, permanent increase of viability has been recorded despite of used concentration of MoLi. After 96 h the viability increased significantly – up to 2782.4 % at the concentration of 1 μM, which is approximately 8 times more than in comparison with the control group.

As result, strong evidences have been acquired for the biological efficiency of these coordination compounds. Therefore, it can be stated that these molybdenum complexes appear non-toxic.

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