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APPLICATION OF EPITHERMAL NEUTRON ACTIVATION ANALYSIS TO INVESTIGATE ACCUMULATION AND ADSORPTION OF MERCURY BY *SPIRULINA PLATENSIS* BIOMASS

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Применение нейтронного активационного анализа
в исследовании аккумуляции и адсорбции ртути
биомассой *Spirulina platensis*

Метод эпитеплового нейтронного активационного анализа использован в исследовании особенностей взаимодействия сине-зеленой микроводоросли *Spirulina platensis* с токсичным металлом — ртутью. Эксперименты проводились в условиях естественного роста клеток при различных концентрациях ионов Hg(II) в питательной среде. Изучена аккумуляция Hg при культивации биомассы *Spirulina platensis* в течение нескольких суток. Установлен характер динамики накопления ртути биомассой спирулины, а также характер роста ее биомассы при различных концентрациях Hg. В кратковременных экспериментах исследован процесс адсорбции ртути биомассой *Spirulina platensis*. Построена изотерма адсорбции в координатах Фрейндлиха. Установлено, что естественная биомасса спирулины может быть использована для очистки сточных вод от ртути при концентрациях порядка 100 мкг/л.

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Application of Epithermal Neutron Activation Analysis
to Investigate Accumulation and Adsorption of Mercury
by *Spirulina platensis* Biomass

Epithermal neutron activation analysis was used to study interaction of blue-green alga *Spirulina platensis* with toxic metal mercury. Various concentrations of Hg(II) were added to cell cultures in a nutrient medium. The dynamics of accumulation of Hg was investigated over several days in relation to *Spirulina* biomass growth. The process of Hg adsorption by *Spirulina* biomass was studied in short-time experiments. The isotherm of adsorption was carried out in Freindlich coordinates. Natural *Spirulina* biomass has potential to be used in the remediation of sewage waters at Hg concentrations ~100 μg/l.

The investigation has been performed at the Frank Laboratory of Neutron Physics, JINR, and at the E. Andronikashvili Institute of Physics of GAS, Tbilisi.

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INTRODUCTION

Mercury and its compounds are widely used in various branches of industry, agriculture, and medicine penetrating the environment in one way or another. A considerable anthropogenic part of the environmental pollution by Hg is contributed by Hg pyrometallurgy, non-ferrous metallurgy, production of chlorine and caustic soda, consumption of fuel, garbage etc. Moreover, the presence of Hg in one amount or another in almost any mining or fuel resources results in its uncontrollable implication into various technological processes.

Mercury is a scattered element. Its mean content in crust constitutes $8{\cdot}10^{-6}\%$. The total amount of mercury in the atmosphere is 10^{-9} g/m^3, in soil — $1{\cdot}10^{-6}\%$, in plants (dry mass) — $25 \cdot 10^{-6}\%$ [1–3].

As for toxicity, mercury holds the first position among other heavy metals and, according to the accepted classification, it belongs to the first group of toxic substances. Even in very small doses it causes gonadotoxic, mutagenic, neurotoxic and embryotoxic effects. Accumulating mainly in kidneys, liver, and spleen, mercury blocks a biochemical activity of protein molecules and low-molecular compounds. The most toxic organic compounds of mercury are those, which have a high lipidsolubility, which contributes to their penetration through membranes and accumulation in vitally important organs.

The character of Hg distribution in the environment contributes to its toxic influence not only on the personnel of the corresponding branches of industry but, frequently, also on the population in general. Medico-biological studies of the last decades showed the gravity of the «mercury hazard» related to the transition of chronic poisoning by Hg vapor from the professional diseases into the disease of population.

Thus, the necessity to study the peculiarities of Hg interaction with living systems is quite obvious, as well as the investigation of biological chain of its migration, owing to which Hg may occur in foodstuff and in feed. In the present paper a blue-green microalgae *Spirulina platensis (S. platensis)*, which is widely used as a basis for pharmaceuticals and also as a biologically active food additive for humans and animals, is considered as a living system.

Hg concentration 0.1 μg/l suppresses the biological activity of algae and at Hg concentration 1 μg/l the processes of photosynthesis and exchange processes are violated [4].

In the process of producing of large amount of *S. platensis*, the biomass is often cultivated in an open pond on the vast area. Here the chance of Hg penetration into the nutrient medium both with atmospheric deposition and with air is rather good, that is why it is a very important task to provide the purity of the *S. platensis* biomass during its production.

On the other hand, algae are often used in remediation of water from heavy metals [5–7]. In this case the methods of binding metals and other toxic elements may vary depending on the type of algae, cultivation conditions, type of toxicants, etc. In certain cases dry algae biomass is used; in others, the interaction with metals occurs in the process of their cultivation *in vivo*. One of the vast and various alga group is cyanobacteria and *S. platensis* is one of them. Cyanobacteria are characterized by high tolerance and can exist in various extreme conditions: in hot springs, in snow, in water rich in salts, etc. Simultaneously, they are very sensitive to the pollution by heavy metals [8]. A possibility to use *S. platensis* dry biomass for remediation of sewage waters from cadmium is shown in [9].

The aim of the present paper is to study the processes of accumulation and adsorption of mercury by the *S. platensis* biomass depending on the Hg concentration in the medium, where the growth of *Spirulina* cells occurs. Scientific and applied interest in the present task is related both with the evaluation of *Spirulina* biomass quality for food and pharmaceutical purposes and with the possibility to use it in remediation of sewage waters from mercury.

1. MATERIALS AND METHODS

Experiments:

To carry out the experiments, we used the *Spirulina platensis* IPPAS B-256 strain from Timiriazev Institute for Plant Physiology of the Russian Academy of Sciences. Cultivation was carried out in a standard Zaroukh alkaline water-salt medium at a temperature of $+34^\circ$ C, illumination ~ 5000 lx, initial pH 8.7 and at constant mixing. Since the Hg(II) ions in alkaline medium form an insoluble residue (mercury hydroxide), in order to avoid the mercury residue, mercury glycinate was used as a nutrient loading.

Preliminary experiments were carried out in order to choose the interval of loading concentrations of alkaline medium by mercury glycinate, at which the cells of *S. platensis* (according to microscopic and spectrometric data) retained their vitality. The experiments showed that at Hg concentrations 16 mg/l the *S. platensis* cells were destroyed completely in 1 hour, at Hg concentrations 1.6 mg/l — in 4 hours, etc. At low concentrations the cells of *Spirulina* were not destroyed but their growth, cytology, pH and spectral characteristics changed essentially.

Based on the results obtained the mercury glycinate doses were determined to be used later on.

1. In the first series of experiments to study the Hg accumulation by the *S. platensis* cells the concentrations of nutrient medium loading by mercury constituted 100, 50, 5, 1, 0.1 μg/l. Initial concentration of *S. platensis* suspension in all the experiments of the series constituted 260 mg/l. Cultivation of the *S. platensis* cells was conducted for 6 days. Samples in all the series were taken every 24 hours. In the course of all the experiments microscopic control of *S. platensis* living cells was performed daily. The protein composition of the biomass was investigated by gel-electrophoresis in sodium dodecyl sulphate polyacrylamide gel (SDS-PAAG).

2. In the second short-term series of experiments to study the Hg adsorption by *S. platensis* cells the initial density of *S. platensis* suspension was relatively higher and constituted 1 g/l. Mercury concentration of nutrient medium loading was 500 μg/l. Dynamics of the adsorption processes, usually taking place during 1–2 hours, were studied during 1 hour. Samples were obtained in 2, 10, 20, 40 and 60 minutes after the beginning of cultivation.

In all the cases the biomass from the obtained samples was separated by filtration, washed with twice-distilled water till pH 6.5.

The resulting samples were lyophilized [10], and then they were made into small pellets with the mass approximately 0.5 g, which were intended for neutron activation analysis.

Analysis:

Mercury content in the samples was determined by epithermal neutron activation analysis (ENAA) at the pulsed fast reactor IBR-2 (FLNP, JINR, Dubna). The description of irradiation channels and pneumatic transport system of the IBR-2 are given in [11]. Earlier we used the technique of ENA analysis of *S. platensis* samples both to determine its background elemental content and to study accumulation processes of some trace elements [12, 13]. Since mercury is a volatile element, the temperature in the sample irradiation channel is of great importance. It is shown in the paper [14], that at mercury detection it is desirable to irradiate biological samples at the temperature not higher than 90–100 °C. One of the IBR-2 channels with cadmium screen, in which the temperature does not exceed 60–70 °C, meets the requirements entirely.

The samples were irradiated for 5 days and their activity was measured twice, in 4 and 20 days.

The mercury content was determined by γ-line with the energy 279.1 keV of isotope ^{203}Hg. Here the influence of interference lines ^{75}Se and ^{182}Ta was taken into consideration.

Quality control of analytical measurements was carried out using certified standards for biological samples — Lichen (IAEA, Lichen 366), Bottom Sediments (IAEA SDM-2T) and Danish Moss (DK-1).

The ENAA data processing and determination of Hg concentrations were performed using software developed at FLNP, JINR [15].

2. RESULTS AND DISCUSSION

1. The results of experiments to study Hg accumulation from nutrient medium by the *Spirulina platensis* biomass at cell cultivation during 6 days at various Hg concentrations are presented in Fig. 1. In all the cases the exponential character of decrease of Hg content is observed. The curves are well approximated by the function $y = y_0 + A\mathrm{e}^{-x/t}$, the corresponding parameters are given in table.

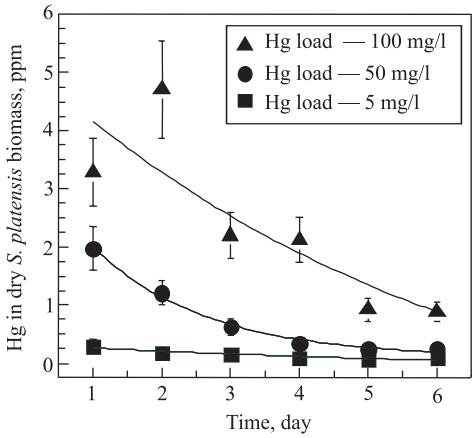

Fig. 1. Hg accumulation from nutrient medium by the *Spirulina platensis* biomass at various loadings during 6 days

Table. **Approximation Parameters of the Exponential Function**

Hg loading, μg/l	100	50	5
χ^2	0.79	0.005	0.001
R^2	0.71	0.99	0.91
y_0	-1.8 ± 1.4	0.11 ± 0.1	-0.12 ± 0.04
A	6.9 ± 2.2	3.5 ± 0.3	0.45 ± 0.07
t	6.2 ± 0	1.6 ± 0.2	6.2 ± 0

Such a character of dependence seems to be clear, as the number of *S. platensis* cells grows exponentially, the number of sites of Hg(II) ion binding

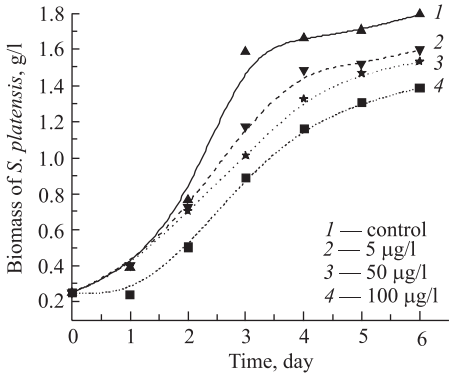

Fig. 2. Growth dynamics of the *S. platensis* biomass at various Hg (II) loadings

surpasses considerably the number of Hg(II) ions in nutrient medium. This results in blocking of toxic Hg ions and their removal from the nutrient medium. Such a mechanism may serve as one of the important ways for the biosphere to «self-purify» from heavy metals with the help of microorganisms.

Dynamics of the *Spirulina* biomass growth at the same Hg concentrations is shown in Fig. 2. As seen from the given curves, the presence of mercury considerably inhibits the cell growth after more than 60 hours since the beginning of cultivation and the Hg influence is stronger at high concentrations. These results are in line with the data of the paper [16].

Ions of metals interact either with negatively charged carboxyl and phosphate groups of cell surface and membrane or penetrate the cell through specific transport channels of penetration, such as the channels Mg^{2+}, Mn^{2+}, and Ca^{2+}. Metals, which penetrated the cell, are to be found in various cell structures, DNA and ribosomes.

Mercury, even at low concentrations, has toxic influence on the processes of photosynthesis in *S. platensis,* which, primarily, gives rise to a change in chlorophyll fluorescence [17]. This is also confirmed by our investigation of absorption spectra of chlorophyll and C-phycocianin in the regions of 680 and 620 nm.

Mechanisms of neutralization and removal of toxicants from cells are specific for different metals, complicated, and not studied well enough. This may be the formation of metallothioneine-like proteins induced by metals, formation of inert compounds of HgS type, precipitation into the nutrient medium of substances, which form complexes with metals during suppression of their toxic influence. Reduction of Hg in the cultivation medium and in the cells of *Chlorella* and *Dunaniella* species was the result of evaporation [18].

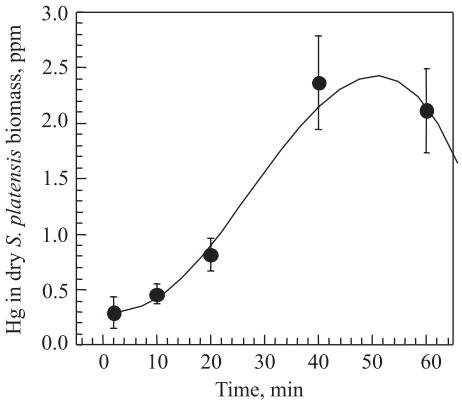

Fig. 3. Hg(II) adsorption by the *Spirulina platensis* cells

2. The results of investigation of Hg adsorption process by the *S. platensis* cells are presented in Fig. 3. The experimental data obtained by the ENAA method are well approximated by the polynomial of the third order: $y = 0.3586 - 0.02286x + 0.00332x^2 - 0.0000406482x^3$. As seen from the obtained curve, the maximum Hg content is adsorbed by the *S. platensis* biomass within 50 minutes and then a diminution of concentration is observed.

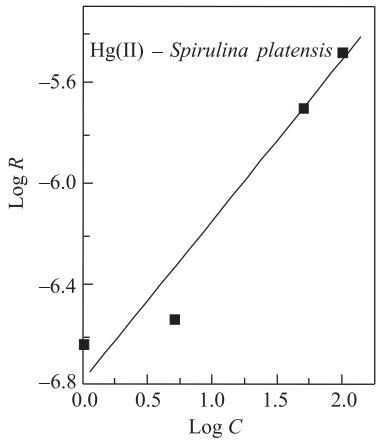

Fig. 4. Freindlich diagram. Linear approximation by method of least squares

A similar character of dependence of Hg(II) accumulation was also obtained in paper [19] during studies of biosorption in other microorganisms.

Theoretical calculations of the adsorption isotherm on the basis of the obtained experimental data were performed in accordance with the Freindlich model, which takes into account both physical adsorption and chemosorption [20, 21]. For various Hg(II) concentrations at a duration of cultivation of 24 h in the Freindlich coordinates the dependence was obtained

$$\log R = -6.77 + 0.62 \log C,$$

where R is concentration of the adsorbed Hg(II), C is concentration of Hg(II) in nutrient medium.

This dependence is a straight line (Fig. 4), cutting off on the Y-axis a section, equal to $\log K$, where K is a constant of biosorption. In the present case $\log K = -6.77$ and $K = 0.17 \cdot 10^{-6}$. The correlation coefficient is $R = 0.97$. The obtained result can be regarded as a confirmation of predominance of biosorptive processes at the initial stage of the *S. platensis* cell cultivation.

If we take into account that Hg content in control samples constituted approximately 0.007 ppm, then it turns out to be that in 50 minutes the *S. platensis* biomass accumulates about 300 times more mercury. Thus, at relatively low Hg concentrations (of the order of 100 μg/l) in the medium *S. platensis* can be used in the remediation of industrial and sewage waters from mercury.

Here, it should be also noted that the *S. platensis* biomass consisting of long trichomes can be easily separated by filtration, which makes the technological process considerably cheaper and simpler.

CONCLUSIONS

1. By the ENAA method it is possible to control the rate of Hg assimilation from nutrient medium by the *S. platensis* biomass in the course of its cultivation in open ponds.

2. At Hg concentrations of the order of 100 μg/l the *S. platensis* biomass in its natural state may be used to accumulate Hg(II) ions for the purpose of their removal from the cultivation medium.

3. The *S. platensis* biomass is suitable for fast remediation of industrial and sewage waters from mercury by way of biosorption and subsequent separation with the help of filtration.

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