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GENERAL BIOLOGY

The Aquatic Macrophyte *Ceratophyllum demersum* Immobilizes Au Nanoparticles after Their Addition to Water

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Study of biogenic migration of elements is an important line of biosphere research [1–8]. Biogenic migration of elements in aquatic ecosystems, both freshwater and saltwater, is important for water self-purification and quality formation [9–11].

Data on element accumulation and binding by aquatic organisms, including macrophytes, are important for analyzing the organisms' important role in biogenic migration of elements in aquatic ecosystems.

The concentrations of some elements were determined earlier in different biogenic samples, including hydrobionts and biogenic detrital substances [9, 10]. However, these data were based on a limited set of organisms and elements. There are no published data on either gold (Au) content in *Ceratophyllum demersum* L. or interaction between Au nanoparticles and aquatic macrophytes.

The goal of this work was to study the immobilization of Au nanoparticles added to the water medium of microcosms in the presence of macrophyte hydrobionts *C. demersum* L.

MATERIALS AND METHODS

Experiments were carried out in freshwater microcosms. *C. demersum* L., a widely spread freshwater plant, was used to form microcosms. Aquatic macrophytes and settled tap water (STW) were added to the microcosms. *C. demersum* plants were collected in a pond located in the floodplain of the upper Moscow River.

After microcosm formation (Table 1), they were incubated under natural photoperiodicity conditions. The water temperature was maintained at 17°C. Nano-size particles (NSPs) of colloidal Au were added to the microcosms.

Obtaining NSPs. Particles were obtained by redox condensation in the water phase [12] using chloroauric acid (Fluka, Germany). 1% $HAuCl_4$ was added to deionized water of a high-degree purity; mixture was brought to the boiling point and agitated, and sodium citrate was added. Boiling was continued, and then the mixture was cooled to room temperature. IgG and bovine serum albumin (BSA, fraction V; Sigma, United States) [12, 13] were used as stabilizing supplements. To obtain the IgG–NSP conjugate, the NSP preparation was mixed with IgG solution, then BSA was added; the particles were separated by centrifugation [13]. IgG–NSP conjugates were resuspended in a 0.01 M potasium phosphate buffer solution, pH 7.0.

The particle size was 20 ± 5 nm. The NSP preparation contained 3×10^{-4} M Au. Two types of macrophyte incubation in medium containing NSP were used.

In first variant (microcosms 1 and 2), nanoparticles without the protein covering were added (without IgG treatment). In the second variant (microcosms 3 and 4), nanoparticles with protein covering were added; i.e., Au NSPs pretreated with rabbit immunoglobulin (IgG, 150 kDa). The added volume was 2 ml. The treatment scheme was as follows: three additions in each microcosm at 24-h intervals. Incubation was over 24 h after the last addition. The total amount of Au added to microcosms 1, 2, 3, and 4 was about 3.6×10^{-6} M. Au NSPs were not added to microcosms 5 and 6 (control).

Table 1. Composition of microcosms containing *C. demersum* macrophytes in 500 ml of STW

System component	1	2	3	4	5	6
C. demersum L. (dry weight), g	7.1	5.8	5.0	5.6	5.7	5.1
Additives, NSP	Au	Au	Au-IgG	Au-IgG	–	–

Note: A dash means a control sample without Au.

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After termination of all incubations, macrophytes were removed from all the six microcosms, dried, and triturated. Neutron activation analysis (NAA) was carried out. We used this method earlier to measure concentration of elements in hydrobionts [11]; this method was proved to be effective for analyzing element contents in samples of biological origin.

Sample preparing and detection of elements were carried out as follows.

Specimens for analysis were dried at 105°C; then, samples in the weight interval from 15 to 25 mg were collected and packed together with samples for comparison (KH, ST-1, SGD-1, FFA, RUS-1, Allende, BCR, etc.) and reference samples in aluminum foil packages.

Samples were placed in an aluminum holder and exposed to radiation from 15 to 20 h in the thermal channel of the nuclear reactor of the Moscow Institute of Engineering and Physics. After radiation treatment, the samples were cooled, repacked in clean ampoules to minimize the background. The activity was measured two or three times (5–7 and 15–30 days after irradiation) using semiconductor (high-resolution) germanium detectors (ORTEC) and NUC-8192, 4096-channels impulse analyzer (EMC, Hungary). Spectrum identification and calculation of element content was carried out in an automated mode as described in [11].

The composition of STW used in microcosm formation was studied by inductively coupled plasma atomic emission spectrometry (AES–ICP) using an ICP spectrometer (ICAP-9000, Thermo Jarrel Ash). The water composition is shown in Table 2.

Table 2. The element composition of water (aquatic medium) microcosms

Element	Concentration (mg/l)
Al	0.06
B	0.01
Ba	0.03
Ca	49.4
Cd	<0.001
Co	<0.001
Cr	<0.01
Cu	<0.001
Fe	0.007
K	2.3
Li	<0.01
Mg	12.6
Mn	0.016
Mo	<0.01
Na	10.7
Ni	<0.002
Pb	<0.005
Si	5.1
Sr	0.14
Ti	<0.001
Zn	<0.001

RESULTS AND DISCUSSION

The results of element determination using NAA are shown in Table 3. It should be noted that the contents of some elements in the *C. demersum* phytomass considerably varied. The content of the studied elements in control samples of *C. demersum* phytomass decreased (averaged data) in the following order: Zn > Sr > Zr > Au > Sc. After incubation in the presence of Au nanoparticles, the Au content became the highest, and the order of elements changed to Au > Zn > Sr > Zr > Sc. Only the Au content increased significantly in *C. demersum* macrophytes from macrocosms 1–4 (Table 3). The contents of other elements (Sr, Sc, Zn, and Zr) in *C. demersum* macrophytes from macrocosms 1–4 did not differ substantially from that in the control variants (5 and 6). These data are in good agreement with the fact that these elements (in contrast to Au) were absent in the additives to microcosms. Thus, the concentrations of these elements served as additional control values.

The mean content of Au in the phytomass of microcosms 3 and 4 did not differ from the Au content in the phytomass of microcosms 1 and 2 (Table 3). This means that Ig treatment had no effect on Au immobilization in the studied macrophytes.

One can calculate the arithmetic mean Au accumulation values in microcosms 1–4 under the conditions of incubation of the plants in the presence of Au nanoparticles. This value was 1204.95 ± 287.69 μg/g dry weight (the standard deviation is indicated). The mean content of Au in the *C. demersum* phytomass of control microcosms (5 and 6) was 2.80 μg/g dry weight. Thus, the Au content in *C. demersum* phytomass after incubation in the presence of Au nanoparticles exceeded the mean level in the control microcosms by a factor of 430.3.

At this stage, we were interested in measurement of the total amount of Au associated with phytomass, including both surface-bound Au and that potentially penetrating into plant tissues. In future, we intend to find out whether the penetration takes place. Our data give no answer to this question. This study continues our research in possibility of using macrophytes to eliminate metals from the water medium with which these plants contact [14]. An increase in the Au content in phytomass proved that the plants absorbed this element from the water medium, because there were no other ways of Au intake.

Estimation of approximate accumulation of immobilized elements by *C. demersum* phytomass on

Table 3. Content of Au and other elements in *C. demersum* phytomass in experimental microcosms 1–6 (μg/g dry mass) using NAA results

Element	Microcosm no. and presence of Au NSPs					
	1 (+Au)	2 (+Au)	3 (+Au-IgG)	4 (+Au-IgG)	5 (–Au)	6 (–Au)
Sr	43	36	79	32	30	25
Sc	0.02	0.03	0.04	0.049	0.023	0.034
Zn	780	620	880	1480	810	1710
Zr	30	27	25	26	20	32
Au	1040.6	1331.0	904.2	1544.0	5.54	0.057

Table 4. Detection of Au content in parts of ecosystems and biogenic samples

Sample	Au content	Reference
Biogenic detritus in microcosms where the following hydrobionts were kept for a long time: *Viviparus viviparus*, *Unio pictorum*, *Ceratophyllum demersum*	from 0.025 to 0.27 (on average, 0.15) mg/kg detritus (dry weigh)	This study
Phytomass of *C. demersum*	On average, 2.80 μg/g dry weigh	The same
Phytomass of *C. demersum* after incubation with NSPs	Increase by a factor of 430.3	The same
Shells of *Unio pictorum*	On average, 0.06 μg/g	According to [11]
Shells of *Viviparus viviparus*	On average, 0.006 μg/g	According to [11]
Marine sediments	1×10^{-8} gmol/kg	On average, according to [7]
Iron and manganese nodules in the world ocean	1×10^{-8} gmol/kg	On average, according to [7]

large areas of ecosystem bottom, where the phytomass may reach 50 g, 500 g or 500 kg dry weight, can be done on the basis of our data. Au accumulation could reach 60.2 mg, 602.5 mg, and 602.48 g, respectively. Our calculations are approximate. They are only intended for estimation of the *C. demersum* phytomass potential for Au accumulation, and it would be incorrect to extrapolate the obtained results to natural ecosystems.

The characteristic feature of our study was that Au immobilization occurred within a specific time by the phytomass of a specific plant, with the studied element added in the NSP form. Phytotoxicity of NSPs during incubation of microcosms containing macrophytes was not observed. However, we have no data to prove definitely the absence of phytotoxicity of Au NSPs.

This study was the first to determine the concentration of Au and some other elements in *C. demersum* phytomass after incubation in microcosms containing Au NSPs.

Thus, (1) the first data have been obtained that demonstrate that a significant amount of Au NSPs may bind to live phytomass of an aquatic plant, *C. demersum*. As a result of binding and/or immobilization, the content of Au was greater then the background level by a factor of 430.3; (2) modification of NSPs with a protein (immunoglobulin) has no effect on NSP immobilization by macrophytes.

The results contribute to understanding the role of these organisms in biogenic migration of elements. These data give us the possibility to estimate the participation of phytomass of the studied macrophyte species in element concentration (as exemplified by Au) in an aquatic system.

Vernadsky had placed special emphasis on "the importance ...of living masses ... as places of the most intense migration of atoms in the biosphere" and highlighted the concentration function as one of the main functions of living matter [1]. Our data on Au immobilization by *C. demersum* phytomass confirm these statements by a specific example. The new results complete published data on the multifunctional role of the biota in the migration of elements and coupling of geochemical and hydrobiological processes [1–11]. Accumulation of new data on Au NSP interaction with aquatic organisms is of interest for studies on the interaction of Au and Au NSPs with cells and organisms, which has medical implications, and for the development of biotechnology of water purification and pathogen detection [15].

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