INTRODUCTION

At present, the biosphere is affected by chemical pollution [1, 2], which manifests itself in both terrestrial and aquatic ecosystems.

Continuous entry of different chemical substances into aquatic ecosystems makes topical problems of ecological and ecotoxicological monitoring, hazard assessment of chemicals, and study of different aspects of the interactions between the chemicals entering the biosphere and organisms [1–5]. In these studies, new aspects of the interactions between pollutants with aquatic macrophytes under the conditions of laboratory microcosms were studied previously [6–9].

This work is devoted to the study of earlier unknown biological effects observed under the influence of gold nanoparticles on aquatic organisms (using macrophytes as the example). Gold (Au) is a heavy metal of the first and six periods of D.I. Mendeleev’s systems of elements with the atomic number 79 and atomic weight of 196.9665 ± 1. The biological effects of this element have been studied less than the influence of other heavy metals [10, 11]. Scientific literature lacked the information about the interactions of Au nanoparticles with aquatic macrophytes, as well as data whether gold nanoparticles can exert toxic action in terms of higher aquatic plants.

The current problems in the study of ecotoxicology and chemical–biotic interactions [12–18] make it necessary to gain scientific information on the potential toxicity of a maximum broad range of chemical substances and products, including nanoparticles.

The aims of this study were to verify the hypothesis on the possible biological activity of gold nanoparticles and reveal if they can exert toxic action on aquatic macrophytes Ceratophyllum demersum L.

MATERIALS AND METHODS

The experiments were carried out in freshwater microcosms. The microcosms were created using abundantly occurring freshwater organisms—aquatic plants Ceratophyllum demersum L. According to the previously worked out method of macrophyte keeping under laboratory conditions [6, 8, 9], aquatic macrophytes and settled tap water (STW) were applied to microcosms. The Ceratophyllum demersum plants were collected in a pond in a floodplain in the upper reaches of the Moscow River.

Macrophytes C. demersum were incubated in microcosms of transparent polymeric material under the conditions of natural photoperiodicity. Each
The state of macrophytes during incubation is characterized in Table 2. During incubation of macrophyte macrocosms in the first days, signs of AuNPs phytotoxicity were not observed.

It was shown that, under the conditions of experiment after the total addition of AuNPs of $1.8 \times 10^{-5}$ M, marked phytotoxicity was observed after 24 days.

At the total addition of AuNPs of $6 \times 10^{-6}$ M, certain signs of phytotoxicity also manifested but to a lesser degree.

We note that, during influence of AuNPs along with death of a portion of shoots, sublethal effects were observed associated with localization of plants’ shots in a column of water. During toxic sublethal influence, the shoots located in the column of water, on average, lower than in the control. In control, all the shoots floated in the column of water and did not touch the bottom and the shoots sank lower and certain shoots touched the bottom during action of AuNPs. We noted similar sublethal effects when observing macrophytes that were incubated in the presence of such heavy metals as Cu, Zn, Cd, and Pb, as well as during incubation of macrophytes in the presence of an organic pollutant (sodium dodecyl sulphate, SDS). This indicates that we found and used a new method for detection and characterization of sublethal manifestations of phytotoxicity during influence of pollutants on aquatic plants C. demersum.

The results supplement the accumulated information on ecotoxicity of metals [1, 2, 4, 5] and phytotoxicity of chemical substances (for example, [3, 6–9]), as well as on chemical–biotic interactions in an aquatic environment [12–18]. New results supplement the previously found facts on phytotoxicity of

| Number of microcosms | Biomass of macrophytes C. demersum (wet weight), g |
|----------------------|---------------------------------------------------|
| 1                    | 4.5                                               |
| 2                    | 3.4                                               |
| 3                    | 4.4                                               |
| 4                    | 3.4                                               |
| 5                    | 1.9                                               |
| 6                    | 2.3                                               |

microcosm contained 500 mL of water (STW) and macrophytes at a quantity corresponding to the biomass of 2–4 g of the wet weight (Table 1). The temperature of water was 20°C. Preparations of colloid nano-sized gold particles of Au (AuNPs) were added to the microcosms. The size of the particles was 20 ± 5 nm. The AuNPs preparation contained $3 \times 10^{-4}$ M Au. The volume added to microcosm nos. 1 and 2 was 2 mL and that added to microcosm nos. 3 and 4 was 6 mL. The mode of AuNPs additions: 5 additions in each microcosm were made. The first addition was made at the beginning of incubation. Following additions were made on the third, eighth, 17th, and 25th days of incubation. After 28 days, the incubation was terminated. The total application of Au into microcosm nos. 1, 2, 3, and 4 after the last fifth addition comprised: in microcosm nos. 1 and 2 after introduction of five additions of 2 mL—$6 \times 10^{-6}$ M; in microcosm nos. 3 and 4 after introduction of five additions of 6 mL—$1.8 \times 10^{-5}$ M. Microcosm nos. 5 and 6 were control samples—nanoparticles were not added.

### Table 1. Biomass of Ceratophyllum demersum macrophytes in microcosms used for incubation in the presence of AuNPs

| Number of microcosms | Biomass of macrophytes C. demersum (wet weight), g |
|----------------------|---------------------------------------------------|
| 1                    | 4.5                                               |
| 2                    | 3.4                                               |
| 3                    | 4.4                                               |
| 4                    | 3.4                                               |
| 5                    | 1.9                                               |
| 6                    | 2.3                                               |

### Table 2. State of C. demersum macrophytes during incubation in the presence of AuNPs

| Number of microcosms | Added dose, mL | Totally added (as calculated to 1 L) | Manifestation of phytotoxicity after 17 days | Manifestation of phytotoxicity after 24 days | Manifestation of phytotoxicity after 28 days |
|----------------------|---------------|-------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| 1, 2                 | 2             | $6 \times 10^{-6}$ M                | Almost all the shoots float; 1–2 shoots touch the bottom | Plants occupy the upper 70–80% of the water column | Area of location of plants in the column of water is broader than in the control; a part of the plants locates lower than in the control. Certain shoots touch the bottom. 20% of the shoots died |
| 3, 4                 | 6             | $1.8 \times 10^{-5}$ M              | Part of the shoots sank to the bottom of microcosms; end parts of certain shoots start to die | Plants occupy the whole column of water; part of shoots touch the bottom or lies on the bottom. Death of 40–50% of shoots is apparent | Plants occupy the whole column of water, including the near bottom area. Part of shoots touch or lies on the bottom. 50% of shoots died |
| 5, 6                 | 0             | 0                                   | All the shoots float in the upper part of microcosms. No shoots touch the bottom | All the shoots float in the upper part of microcosms. The area of macrophyte location is higher than in vessels 1–4. No shoots touch the bottom. Less than 10% of the shoots died | All the shoots float in the upper part of microcosms. The area of macrophyte location is higher than in vessels 1–4. No shoots touch the bottom. Less than 10–15% of the shoots died |
The examples of phytotoxicity of different chemical substances and preparations are given in Table 3. We note that some of these substances are membranotropic and can influence the structure and functions of biological membranes.

These results contribute to the study of the interactions of metals with aquatic plants [34, 35], as well as to study of toxicity of nanomaterials for aquatic organisms [36]. The accumulated facts on a potential toxicity of nanomaterials are of interest because nanomaterials are proposed for use in decontamination of water [37].

The fields of possible use of the results are given in Table 4 [38, 39].

This study leads to the following conclusions.

### CONCLUSIONS

1. A hypothesis was suggested and verified that nanomaterials represented by gold nanoparticles can...
exert phytotoxicity to aquatic environment. Data have been obtained for the first time that gold nanoparticles (Au) under certain conditions have a toxic influence on aquatic macrophytes.

(2) Phytotoxicity under the conditions of laboratory macrocosms was shown. Under the conditions of the experiment in microcosms, toxic influence of gold nanoparticles (Au) on macrophyte C. demersum was established.

(3) In the conditions of the experiments, the toxic influence of gold nanoparticles manifested after quite long-term exposure during 17 days and more.

(4) Phytotoxicity was detected at a concentration of gold (in the form of nanoparticles) of $6 \times 10^{-6}$ M–$1.8 \times 10^{-5}$ M. That phytotoxicity can also manifest at different concentrations can also not be excluded. When increasing the concentration of the Au nano-sized particles (AuNPs) to $1.8 \times 10^{-5}$ M, phytotoxic effects manifested earlier than at the total concentration of $6 \times 10^{-6}$ M.

(5) This study expands the methodological arsenal of biotesting. A new efficient method for phytotoxicity assessment of water soluble or substances suspended in water was successfully approved in this study, which includes analysis of the location of macrophytes’ shoots (using C. demersum as example) in a column of water.

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