ISSN 0096-3925, Moscow University Biological Sciences Bulletin, 2014, Vol. 69, No. 3, pp. 108–112. © Allerton Press, Inc., 2014.
Original Russian Text © S.A. Ostroumov, V.A. Poklonov, S.V. Kotelevtsev, S.N. Orlov, 2014, published in Vestnik Moskovskogo Universiteta. Biologiya, 2014, No. 3, pp. 19–23.

ECOLOGY

Toxicity of Gold Nanoparticles for Plants in Experimental Aquatic System

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Received April 10, 2013

Abstract—Increased production and use of nanomaterials can lead to new types of pollution of the environment, including aquatic ecosystems. Pollution of the aqueous environment with nanoparticles can be a new type of pollution of the environment. This requires a more detailed study of the biological effects during exposure of nanoparticles on aquatic organisms. The interactions of gold nanoparticles (Au) with aquatic macrophytes *Ceratophyllum demersum* have been studied. Aquatic microcosms with these plants were used. Gold nanoparticles (Au) were added to the aqueous medium of *C. demersum* macrophyte containing microcosms. The state of the plants was then analyzed. Phytotoxicity of Au nanoparticles for aquatic macrophytes was shown for the first time. A new method of phytotoxicity detection was suggested and successfully approved. Phytotoxicity at a concentration of Au (in the form of nanoparticles) of 6×10^{-6} M–1.8×10^{-5} M was shown.

Keywords: hydrobionts, aquatic microcosms, macrophytes, nanoparticles, Au, *Ceratophyllum demersum*.

DOI: 10.3103/S0096392514030080

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].

In connection with the development of the possibilities of using gold nanoparticles (AuNPs) for medical purposes, including for diagnostic and therapy of cancer, Alzheimer's disease, arthritis, HIV, and tuberculosis [11], it is of interest to study the whole spectrum of possible biological effects caused by different Au preparations. Certain Au (I) preparations are toxic; they accumulate in the kidneys and, to a less degree, in the liver, spleen, and hypothalamus; accumulation in the kidneys can lead to diseases of the kidneys, as well as to dermatitis, stomatitis, and thrombocytopenia [10]. 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

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

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 3×10^{-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—6×10^{-6} M; in microcosm nos. 3 and 4 after introduction of five additions of 6 mL—1.8×10^{-5} M. Microcosm nos. 5 and 6 were control samples—nanoparticles were not added.

RESULTS AND DISCUSSION

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 1.8×10^{-5} M, marked phytotoxicity was observed after 24 days.

At the total addition of AuNPs of 6×10^{-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

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

Number of micro-cosms	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	6×10^{-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	1.8×10^{-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

Table 3. Phytotoxicity of inorganic and organic substances, examples

Chemical substances	Organisms	References
Gold nanoparticles	Aquatic macrophytes *Ceratophyllum demersum*	New results in this paper
Surface-active substances (SASs) sodium dodecyl sulphate, Triton X100, tetradecyltrimethylammonium bromide	Seedlings of plants of *Fagopyrum esculentum*, *Sinapis alba*, *Oryza sativa*	[26]
Cationic SAS	Seedlings of *Fagopyrum esculentum*	[27]
Polymeric SAS	Seedlings of *Fagopyrum esculentum* Moench	[28]
Sodium dodecyl sulphate	Aquatic macrophytes *Najas guadelupensis* L.	[9]
SAS	Seedlings of plants of *Sinapis alba*, *Triticum aestivum*	[29]
SAS sodium dodecyl sulphate	Aquatic macrophytes *Potamogeton crispus* L.	[30]
Liquid detergent Vilva	Seedlings of plants of *Fagopyrum esculentum*, *Oryza sativa*	[26, 31]
Aist-Universal Synthetic detergent	Aquatic macrophytes *Fontinalis antipyretica* Hedw.	[6]
Kashtan Detergent	Aquatic macrophytes *Pistia stratiotes*	[26]
Kristall Synthetic detergent	Seedlings of plants of *Oryza sativa*, *Fagopyrum esculentum*	[26]
Kashtan Detergent	Seedlings of plants of *Oryza sativa*, *Cucumis sativus*	[26]
Aist-Universal Synthetic detergent	Seedlings of plants of *Fagopyrum esculentum*	[7]
Heavy metals (Cu, Zn, Pb, Cd)	Aquatic macrophytes *Lilaeopsis brasiliensis* and *Utricularia gibba*	[32]
Nanoparticles of metal oxides (TiO_2, CuO, Al_2O_3)	Seedlings of plants of *Lens culinaris*	[33]
Nanoparticles of copper oxides	Aquatic macrophytes *Elodea Candensis*	[24]

Table 4. Possible use of the results of this work

Area of application	How this work is useful in this area, how the results can be applied
Methodology of scientific research in the field of nanomaterials	Inexpensive and efficient method for assessing the potential hazards of nanoparticles was developed and approved
Methodology of biotesting of potentially toxic substances	New, easy-to-use, practical method for estimating phytotoxicity of substances was developed and approved using aquatic macrophytes and microcosms
Science of nanomaterials	New type of nanomaterials exerting phytotoxicity was identified (gold nanoparticles) and the database of metal nanoparticles was expanded
Prediction of the properties of new substances and materials	New data useful for predicting the properties of substances and materials with regard to their degree of dispersion were obtained. The range of specific examples was expanded where increased dispersion of nontoxic substances leads to the appearance of a new property—toxicity
Environmental education, environmental safety	Range of facts about substances that can act as toxic pollutants of the environment with harmful effects on living organisms was expanded. It can be used for a more complete assessment of environmental impacts (AEI)

chemical substances for higher plants detected when plant seedlings were tested (for example, [26–29, 31, 33]) and on higher aquatic plants (for example, [9, 24, 30, 32]).

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 6×10^{-6} M–1.8×10^{-5} M. That phytotoxicity can also manifest at different concentrations can also not be excluded. When increasing the concentration of the Au nanosized particles (AuNPs) to 1.8×10^{-5} M, phytotoxic effects manifested earlier than at the total concentration of 6×10^{-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.

ACKNOWLEDGMENTS

We thank Yu.A. Moiseeva, E.A. Solomonova, colleagues of Moscow State University and Vernadsky Institute of Geochemistry and Analytical Chemistry, Russian Academy of Sciences, for their aid and discussion.

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Translated by M. Novikova