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Abstract—Copper is one of the key heavy metals that pollute environment and constitute a serious threat to the health of humans and ecosystems. Copper may enter the aquatic environment in both soluble and nanoparticle form. It was previously found in a series of studies that nanoparticles, including those of several metal oxides, exercise both negative and positive effects on the higher plants which makes necessary further research on the interaction between metal oxide nanoparticles and plants. Interactions between aquatic plants and copper–containing nanoparticles were not sufficiently studied. The goal of this study was to contribute to the investigation of the interactions between CuO nanoparticles and the aquatic plant *Elodea canadensis* under the conditions of experimental microcosms. It was found that CuO nanoparticles demonstrated some phytotoxicity to *Elodea canadensis*. After the incubation of *Elodea canadensis* in the aquatic medium contaminated with CuO nanoparticles there was a significant increase (by two orders of magnitude) of the concentration of copper in the biomass of the plants.
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INTRODUCTION

Copper is one of the key heavy metals that pollute the environment and constitute a serious threat to the health of humans and ecosystems [1–3]. Copper may enter the aquatic environment in both soluble and nanoparticle form.

It was found in a series of studies that nanoparticles including those of several metal oxides, exercise both negative and positive effects on higher plants [4–7] which makes necessary further research on the interactions between metal oxide nanoparticles (NP) and plants. Binding of several types of NPs to terrestrial plants has already been found [5]. By contrast, no binding of Fe oxide NPs to some species of higher plants (*Phaseolus limensis*) has been detected [8].

Studies of interaction of pollutants with aquatic plants are of significant importance to both fundamental ecology and applications [9–11]. The applications include environmental monitoring and remediation of polluted freshwater ecosystems. Among various types of aquatic pollution, metal pollution is one of the most important [12–17], which makes it urgent to continue very active studies of various forms of metal aquatic pollution (including pollution from NPs that contain heavy metals) and its interaction with freshwater macrophytes.

Interactions between freshwater plants and copper–containing NPs have not been studied sufficiently. The goal of this study was to contribute to the investigation of the interactions between CuO NPs and the aquatic plant *Elodea canadensis* (below *E. canadensis)* under conditions of experimental microcosms.

EXPERIMENTAL PART

Methods. The plants of *E. canadensis* were selected for studies due to the following reasons: they represent

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Table 1. ICP–OES operating conditions and parameters for data acquisition

Parameters	Units	Numerical value
RF (Radio Frequency), Power	W	1300
Gas Flow Rate	L min^{-1}	–
Plasma	L min^{-1}	15.0
Auxiliary	L min^{-1}	0.2
Nebulizer	L min^{-1}	0.8
Reading/Replicates	–	1.0
Integration Minimum	s	1.0
Integration Maximum	s	5.0
Read Delay	s	30.0
Replicates	–	5.0
Wash Time	s	120.0
Flush Time	s	8.0

one of the key species of aquatic macrophytes with an extremely broad range; this species was used in previous studies of accumulation and phytotoxicity of chemical pollutants, which provide material for comparative analysis; this species has outstanding economical and ecological importance as one of aggressive introduced species with a strong potential for invasion into new aquatic bodies.

The plants *E. canadensis* were collected in a wetland next to the University of Massachusetts, Amherst, the sport facilities. The plants were washed and incubated in glass 1L beakers.

The total duration of the incubation was 12 days. During incubation, the photoperiodicity regime was: 14 h at light time (8 a.m.–10 p.m.), 10 h at dark time (10 p.m.–8 a.m.). The illumination was at the level of 18.9 microEinstein m^{-2} min^{-1} measured by Quantum Radiometer Photometer LI–250A Light Meter (photosynthetically active radiation, 400–700 nm). Temperature was 21°C.

The NPs of CuO were used. Concentration of added NPs was 38 mg L^{-1}. This concentration was chosen because it is high enough to observe some effects; however, it is still not very high, which is important as an excessive concentration may be associated with some additional side effects that may make the interpretation of the experiment more obscure. The control microcosms contained no added CuO NPs.

The microcosms were incubated in glass beakers with 500 mL of settled tap water each. The size of the plants was: *E. canadensis* 15–17 cm; *Myriophyllum aquaticus* (below *M. aquaticus*) 16–19 cm. The beakers contained 2 *E. canadensis* plants and 2 *M. aquaticus* plants.

Detection of elements. Trace elements were determined with a PerkinElmer® DV 4300 inductively coupled plasma optical emission spectrometer (ICP–OES, USA). The instrumental operating parameters are listed in Table 1. Parameters such as flow rates, power, and integration times were as recommended by the manufacturer. Prior to analysis of each sample, a flush time of 8 s was chosen to ensure that the plasma reached a steady state. Attention was paid to the display window to assure that the signals of all elements returned to signals close to that of the blank prior to analyzing the subsequent sample. Five replicates per sample were essential for assessing the variability of each digest.

The wavelengths used in detecting of chemical elements were: for Co λ = 228.616 nm; for Cr λ = 267.716; for Cu λ = 327.393 nm; for Mg λ = 285.213 nm; for Ni λ = 231.604 nm; for Ti λ = 334.940 nm; for Zn λ = 206.200 nm.

Sample preparation. For the analysis of the water solutions in each microcosm 2 ml portions were dispensed into microwave digestion vessels in triplicate and 4 ml concentrated 70% solution HNO_3 was added. The digestion vessels were placed into the microwave–assisted reaction system (MARS with Xpress Technology, CEM Corporation, USA) and underwent digestion under the following conditions: samples were ramped to a temperature of 140°C over 20 min and held at that temperature for 30 min under a power setting of 1200 W. Sample vessels were cooled for 1 h and allowed to slowly vent for 20 min. They were diluted to 25 ml in a calibrated volumetric flask with deionized water and stored in a refrigerator until analyzed.

The samples of phytomass (biomass) of plants were oven–dried to a constant weight at a temperature of 55°C. After drying samples were transferred and ground in individual crucibles. 50 mg of each material was weighed out into separate digestion vessels. These samples were left overnight to predigest in a 70% solution of HNO_3. The digestion vessels were placed into the microwave–assisted reaction system and underwent digestion under the same conditions as mentioned for the aqueous samples. Samples were cooled for 1 h and allowed to slowly vent for 20 min. They were diluted and stored under the same conditions written above until analysis.

Table 2. Concentrations of elements in the phytomass of *E. canadensis* in the control microcosms

Element	Concentration of elements in phytomass (dry weight of phytomass)$^{-1}$		
	Microcosm 1	Microcosm 2	Average
Co	3.6×10^{-6}	2.9×10^{-6}	3.3×10^{-6}
Cr	9.5×10^{-6}	1.21×10^{-5}	1.08×10^{-5}
Cu	2.92×10^{-4}	3.39×10^{-4}	3.16×10^{-4}
Mg	1.37×10^{-3}	1.53×10^{-3}	1.45×10^{-3}
Ni	1.13×10^{-5}	1.30×10^{-5}	1.22×10^{-5}
Ti	1.64×10^{-5}	2.28×10^{-5}	1.96×10^{-5}
Zn	1.04×10^{-4}	1.43×10^{-4}	1.23×10^{-4}

Reagents and standards. 70% solution HNO_3 (Fisher Scientific, USA.; certified by ACS) was utilized for cleaning glassware, preparing standard solutions, and digesting samples. Stock multielement standard calibration solutions were prepared using a multielement plasma emission standard.

Quality Control Standard 21 (Perkin Elmer Pure, USA) containing 100 mg L^{-1} of each of elements.

The NPs used were: CuO: Aldrich (Sigma–Aldrich Co., USA); FW 79.54; < 50 nm.

RESULTS AND DISCUSSION

Effects of the nanomaterial on the plants. After 12 days of incubation in the presence of CuO NPs some visible signs of phytotoxicity developed. The leaves of *E. canadensis* became less vigorous, the color less bright. Those symptoms were similar to what we observed in our previous experiments studying phytotoxic effects of another pollutant, a synthetic surfactant [9]. During the following days of incubation, the leaves were darker and darker, possibly as a result of the summation of two effects: mortification of the tissues and sorption of the NPs, which were dark grey, almost black or extremely dark grey–brownish in color. The plants *E. canadensis* gradually lost turgor; the leaves were dark grey to almost black.

It is noteworthy that simultaneously the same treatment with CuO NP was given to the plants of *M. aquaticum*, which did not show any visible signs of phytotoxicity during the same period of time.

Concentrations of chemical elements in the plants: effect of the addition of the nanomaterial. After 12 days of incubation, the plants *E. canadensis* were removed from the aquatic medium, gently washed and taken for chemical analysis. The results of the analysis are presented in Table 2. It is seen that the order of decreasing concentrations of elements was: Mg > Cu > Zn > Ti > Ni > Cr > Co.

In the microcosms with added CuO NPs the concentration of Cu in the phytomass was higher than in the control microcosms (Table 3). It is seen that in the microcosms with added CuO NPs, the measured concentration of Cu in the phytomass exceeded the concentration of Mg and several other measured metals. As a result, the order of decreasing concentrations of elements was:

Cu > Mg > Zn > Ti > Ni > Cr > Co.

To make the comparison of the concentrations in the phytomass of the control and experimental microcosms more quantitative, the ratio (B/A) 100% was calculated and presented in Table 4. It is seen that in the microcosms with the NPs, the concentration of Cu in the biomass (phytomass) increased manyfold as compared to the biomass of the plants from the control microcosms. The concentration of Cu in the phytomass in the experimental microcosms was two orders of magnitude above those in the control ones.

By contrast, the concentrations of the other elements (Co, Cr, Cu, Mg, Ni, Ti, Zn) did not increase in the phytomass of the control microcosms, which is in agreement with the conclusion that the observed increase in Cu is associated with the addition of the CuO NPs to aquatic medium.

There are several ways to explain the results, including possible involvement of a water–soluble form of copper, sorption of copper or copper–containing NPs, and their intake inside plant tissues. The issue of whether

Table 3. Concentrations of elements in the phytomass of *E. canadensis* in the experimental microcosms with CuO NPs

Element	Concentration in phytomass (dry weight of phytomass)$^{-1}$		
	Microcosm 1	Microcosm 2	Average
Co	1.7×10^{-6}	2.7×10^{-6}	2.2×10^{-6}
Cr	6.5×10^{-6}	7.2×10^{-6}	6.9×10^{-6}
Cu	3.74×10^{-2}	3.64×10^{-2}	3.69×10^{-2}
Mg	1.36×10^{-3}	1.50×10^{-3}	1.43×10^{-3}
Ni	8.5×10^{-6}	9.8×10^{-6}	9.2×10^{-6}
Ti	5.1×10^{-6}	2.07×10^{-5}	1.29×10^{-5}
Zn	1.27×10^{-4}	1.44×10^{-4}	1.36×10^{-4}

Table 4. Concentrations of elements in the phytomass of *E. canadensis* in the experimental microcosms with CuO NPs as percentage of the average values in the control

Element	Concentration, (dry weight of phytomass)$^{-1}$ (A)	Concentration, (dry weight of phytomass)$^{-1}$ (B)	Concentration (B), % of the control (A)	Conclusion on whether there is some increase in B relative to A
	A, control	B, with CuO NPs	(B/A)·100	
Co	3.3×10^{-6}	2.2×10^{-6}	66.7	No
Cr	1.08×10^{-5}	6.9×10^{-6}	63.9	No
Cu	3.16×10^{-4}	3.69×10^{-2}	11677.2	Yes
Mg	1.45×10^{-3}	1.43×10^{-3}	99.6	No
Ni	1.22×10^{-5}	9.2×10^{-6}	75.4	No
Ti	1.96×10^{-5}	1.29×10^{-5}	65.8	No
Zn	1.23×10^{-4}	1.36×10^{-4}	110.6	No significant increase

a water soluble form of metals (ions of metals) may be involved in interactions between NPs and organisms is being actively discussed in the literature. Currently it is not possible to exclude this possibility among other possible mechanisms of interaction of NPs with organisms and tissues of aquatic plants. On the other hand, in the current literature, there is evidence on binding of NPs to bacteria and bacterial components, e.g. bacterial polysaccharides [18], and bacterial pili [19], as well as to plants [8, 10], which makes it possible that at least some of the added NPs bind to the plants used. The interpretation is possible that the apparent increase in the metal content of the phytomass may be considered as binding or immobilization of NPs, or immobilization of copper. The current data do not make it possible to discriminate between several possible mechanisms, which makes further studies necessary.

The temporary conclusion is that some amount of copper of the added CuO NPs finally was immobilized on and/or in the phytomass, which means that the corresponding amount of copper was removed from the water column.

The observation of immobilization of copper from the copper–containing NPs on the plant biomass is in agreement with some other facts obtained in the studies of interaction of manufactured NPs with plants.

Table 5. Some examples of the NPs of metals immobilization by plants

Types of NPs	Plants	Detection of binding or immobilization of metal (+) or absence of binding/ immobilization (–)	References
Fe oxide	*Phaseolus limensis*	–	[8]
Au	Aquatic macrophytes	+	[10]
Au	*Spartina alterniflora*	+	[11]
Au; oxides of Al, Fe, Cu, Ti	Aquatic macrophytes *Myriophyllum aquaticum*	+	Ostroumov, Johnson, Tyson, Xing, in prep.
Au; oxides of Al, Fe, Cu, Ti	Litter of aquatic plants	+	Ostroumov, Johnson, Tyson, Xing, in prep.
CuO	*Elodea canadensis*	+	This study

The examples of the current data on how addition of NPs may lead to immobilization of metals by plants are given in Table 5.

The results contribute to better knowledge and understanding of the migration and immobilization of copper in the multicomponent aquatic media and ecosystems. The potential areas of future application of this knowledge are several, including issues of heavy metal pollution and fate in the environment, as well as environmental monitoring and remediation.

Heavy metal pollution and fate in the environment. The fate of heavy metals after entering the aquatic environment was a matter of extensive study and dispute [12–14]. One of the key components of aquatic ecosystems is the biomass of aquatic macrophytes. The previous studies mainly concerned the fate of copper that entered the system in water–soluble form. As for metal–containing nanoparticles, the current knowledge is more about binding to plants. Thus it was observed that ZnO nanoparticles adsorbed onto the terrestrial plant, ryegrass *Lolium perenne* [5]. Fe oxide NPs adsorbed onto pumpkin plants but were not absorbed on another plant species, lima bean (*Phaseolus limensis*) [8]. There was some limited information on binding to aquatic plants. Thus, it was demonstrated that Au NPs were capable of binding to *C. demersum* [10], and to *Spartina alterniflora* [11]. Our new data showed that it is possible to consider the biomass of aquatic plants an additional sink for copper when it enters the aquatic environment in nanoparticle form. We can predict that for other heavy metals as well and may expect to find more facts about that in future.

Environmental monitoring. Aquatic plants are considered specific objects and tools for the monitoring of the state of aquatic ecosystems. It is a well–established fact that water pollution with copper and other heavy metals in form of water–soluble chemicals leads to an increase in concentrations of the those metals in the biomass of aquatic macrophytes [20, 21], which makes it possible to evaluate the degree of aquatic pollution on the basis of measurement of the concentrations of metals in aquatic plants. However next to nothing was known about the potential accumulation of heavy metals in aquatic plants following aquatic pollution with nanoparticles. The new results showed that aquatic plants could be used for purposes of environmental monitoring in case of aquatic pollution with nanoparticles, such as CuO NPs.

Phytoremediation. It is a well–documented fact that plants can accumulate heavy metals and serve a tool for ecotechnologies of cleaning their environment, including the aquatic environment. However, those observations and conclusions were made by studying the systems where the polluting heavy metal was introduced to soil or water in water–soluble form. The majority of research in the area of phytoremediation was done with terrestrial plants. Much less was done with aquatic plants [12–14], and almost nothing about the remediation of environments polluted with NPs. The metal that is associated with plant biomass is removed from the immediate environment of the plant, which in case of our experiments was the aquatic medium. Therefore, the demonstration of binding of copper to the biomass of aquatic plants (the phytomass of *E. canadensis*) contributes to a scientific basis for the technologies of phytoremediation of the aquatic medium contaminated

with copper–containing nanomaterials, as well as to broader knowledge of the fate of nanoparticles in the aquatic environment [15–17].

CONCLUSIONS

It was found that CuO nanoparticles demonstrated some phytotoxicity to the aquatic plants *E. canadensis* under the experimental conditions used (in microcosms).

It was found that after the incubation of the plants *E. canadensis* in the aquatic medium contaminated with CuO nanoparticles, there was a significant increase (by two orders of magnitude) of the concentration of copper in the biomass of the plants.

The new data contributed to a better understanding of the heavy metal's (copper's) fate in the aquatic environment, to extending the scientific basis for environmental monitoring and remediation, and to developing the environmental toxicology of nanomaterials [22].

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REFERENCES

1. Han, B.–C. and Hung, T.–C., *Environ. Pollution*, 1990, vol. 65, no. 4, p. 347.
2. Totaro, E.A., Pisanti, F.A., Glees, P. and Continillo, A., *Marine Environ. Res.*, 1986, vol. 18, no. 4, p. 245.
3. Claveri, B., Morhain, E. and Mouvet, C., *Chemosphere*, 1994, vol. 28, no. 11, p. 2001.
4. Lin, D. and Xing, B., *Environ. pollution*, 2007, no. 150, p. 243.
5. Lin, D. and Xing, B., *Environ. Sci. Technol.*, 2008, no. 42, p. 5580.
6. Ostroumov, S.A. and Poklonov, V.A., *Water: technology and ecology*, 2009, no. 3, p. 38.
7. Musante, C. and White, J.C., *Environ. Toxicology*, 2011, in press.
8. Zhu, H., Han, J., Xiao, J.Q. and Jin Y., *J. Environ. Monit.,* 2008, vol. 10, no. 6, p. 713.
9. Solomonova, E.A. and Ostroumov, S.A., *Moscow University Biol. Sci. Bull.*, 2007, vol. 62, no. 4, p. 176.
10. Ostroumov S.A. and Kolesov G.M., *Doklady Biol. Nauk*, 2010, vol. 431, p. 124.
11. Ferry, J.L., Craig, P., Hexel, P., Sisco, P., Frey, R., Pennington, P.L., Fulton, M.H., Scott, I.G., Decho, A.W., Kashiwada, S., Murphy, C.J. and Shaw, T. J., *Nature Nanotechnol.*, 2009, no. 4, p. 441.
12. Singh, S.K. and Subramanian, V., *CRC Critical Reviews Environ. Control.*, 1984, vol. 14, no. 1, p. 33.
13. Rai U.N., Sinha, S., Tripathi, R.D. and Chandra, P., *Ecol Engineering*, 1995, vol. 5, no. 1, p. 5.
14. Ostroumov, S.A. and Shestakova, T.V., *Doklady Biol. Nauk*, , 2009, vol. 428, no. 1, p. 444.
15. Lin, D., Tian, X., Wu, F. and Xing, B., *J. Environ. Qual.,* 2010, no. 39, p. 1896.
16. Blaser, S.A., Scheringer, M., Macleod, M. and Hungerbühler K., *Sci. Total Environ.*, 2008, no. 390, p. 396.
17. Kaegi R., Ulrich A., Sinnet B., Vonbank R., Wichser A., Zuleeg S., Simmler H., Brunner S., Vonmont H., Burkhardt M. and Boller M., *Environ. Pollut.*, 2008, vol. 156, no. 2, p. 233.
18. Lin, C.–C., Yeh, Y.–C., Yang, C.–Y., Chen, C.–L. Chen, G.–F., Chen, C.–C. and Wu, Y.–C., *J. Am. Chem. Soc.*, 2002, no. 124, p. 3508.
19. Bromberg, L., Chang, E.P., Alvarez–Lorenzo, C., Magarinos, B, Concheiro, A. and Hatton, T.A., *Langmuir*, 2010, vol. 26, no. 11, p. 8829.
20. Samecka–Cymerman, A. and Kempers, A.J., *Water, Air, & Soil Pollution*, 2003, vol. 145, no. 1–4, p. 139.
21. Kähkönen, M.A. and Manninen, P.K.G., *Chemosphere*, 1998, vol. 36, no. 6, p. 1381.
22. Ostroumov, S.A. and Kotelevtsev, S.V., *Ecologica*, 2011, vol. 18, no. 61, p. 3.