Ecotoxicology and Environmental Safety 148 (2018) 152–159

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Urea increased nickel and copper accumulation in the leaves of *Egeria densa* (Planch.) Casp. and *Ceratophyllum demersum* L. during short-term exposure

Maria Maleva, Galina Borisova, Nadezhda Chukina, Adarsh Kumar*

Department of Experimental Biology and Biotechnology, Institute of Natural Sciences and Mathematics, Ural Federal University, Mira str., 19, Ekaterinburg 620002, Russia

ARTICLE INFO

Keywords:
Metals
Bioconcentration factor
Photosynthetic pigments
Oxidative stress
Pro- and antioxidant reactions
Phytoextraction

ABSTRACT

In the present study, two fresh water plant species *Egeria densa* (Planch.) Casp. and *Ceratophyllum demersum* L. were subjected to separate and combined action of urea (2 mM) and metals (Ni and Cu, 10 μM) to investigate the phytoremediation potential of these two submerged macrophytes during short-term experiments (48 h). Both submerged macrophytes demonstrated high accumulative potential for Ni and Cu (average bioconcentration factors were 2505 for Ni and 3778 for Cu). The urea (2 mM) was not significantly toxic for studied plant species. Futhermore, urea worked as an additional source of nitrogen and stimulated some metabolic processes such as the synthesis of photosynthetic pigments, soluble proteins, non-enzymatic antioxidants, and activated some enzymes. Adding urea to the metals increased their accumulation in both macrophytes (on average by 35% for Ni and 15% for Cu). Combined action of urea and Ni did not have a significant effect on antioxidant response, but caused a sharp increase of urease activity (4 folds on an average) in both plants. The copper exerted a stronger toxic effect on both studied macrophytes compared to nickel. Adding urea to copper in some cases diminished the toxic action of this metal. Study concludes that the responses of *E. densa* and *C. demersum* to urea and metal action (separate and combined) were depended on the type of pollutant and the activity of antioxidant defence system. Therefore, the studied aquatic macrophytes found to be potential phytoremediators of water bodies, the addition of an organic nitrogen source in the form of urea in environmentally relevant concentration will increase the efficiency of phytoextraction of metals.

1. Introduction

Metals are one of the major environmental contaminants throughout the world, including Russia. It is well known that aquatic macrophytes are capable to bioaccumulate and bioconcentrate metals several folds greater than their surroundings (Bonanno et al., 2017; Borisova et al., 2016; Shah and Reddy, 2014). Therefore some water weeds are successfully used in phytoremediation technologies (Abdallah, 2012; Goulet et al., 2005; Rai, 2009). Nickel and copper are essential elements required in trace amount for plants, being involved in many metabolic processes. Nickel acts as a key element for some important enzymes including urease, which helps in catalysis of urea to ammonia and bicarbonate by the process of hydrolysis (Chen et al., 2009). It also plays a substantial role in stabilization of ribosome structure and participates in providing nitrogen to plant tissues (Polacco et al., 2013). Copper plays a crucial role in signaling of transcription, oxidative phosphorylation and iron mobilization (Yruela, 2009).

Despite their key role in plant metabolism, the range of these metal concentrations favorable for an optimal growth is very narrow. When these limits are exceeded, the copper and nickel can be very toxic and exerts detrimental effect on plants. These effects manifested alterations in germination, growth processes, plant biomass, disturbs the balance and accumulation of macronutrients (Matraszek et al., 2016; Seregin and Kozhevnikova, 2006). It has been reported that Ni can cause leaf chlorosis and disruption of photosynthesis due to their interference with other essential metal ions or non-directional induction of oxidative stress (Sreekanth et al., 2013). In addition to the toxic effect on the growth, nickel may induce changes in plant leaf anatomy, size and ultrastructure of chloroplasts (Upadhyay and Kumar, 2009). Oxidative stress due to copper treatment has been clearly reported in plants (Chen et al., 2015; Drążkiewicz et al., 2004). These can directly generate reactive oxygen species (ROS) via the Fenton reactions, can exchange essential metals from enzyme active centers or can bind with sulfhydryl groups, cause visible toxic symptoms in plants (Maksymiec, 1997). In addition, excess copper adversely affects the Rubisco activity (Sheoran et al., 1990) and photosynthetic pigments (Bibi and Hussain, 2005).

Among the various organic pollutants that greatly enter water

* Corresponding author.
E-mail address: adarsh.biorem@gmail.com (A. Kumar).

http://dx.doi.org/10.1016/j.ecoenv.2017.10.012
Received 23 June 2017; Received in revised form 2 October 2017; Accepted 4 October 2017

bodies, urea (or carbamide) is the most common one. It comes in aquatic ecosystems from both natural and anthropogenic sources (generally 0.25–1.0 mg urea-N L^{-1}) (Huang et al., 2017). Urea is an endogenous product of mammalian metabolism, also produced by plants, bacteria and fungi as a product of binding ammonia during protein dissimilation (Witte, 2011). Finally, urea releases into the environment due to the decomposition of dead organisms with the subsequent degradation by microbiological processes (Thoren, 2007). Ammonium as the final product of urea hydrolysis is not only toxic for plants but also deteriorates water quality (Zhou et al., 2017). High concentration of urea may cause physiological disorder in plants including detrimental effect on productivity (Krogmeier et al., 1989). Urea ability to cause oxidative stress in plants has been less studied (D'Apolito et al., 2010; Krogmeier et al., 1989). Our previous data showed a significant increase of urease activity and decrease of photosynthetic function in the leaves of *Elodea densa* (an invasive species commonly used in aquariums), when treated with high concentration (500 mg L^{-1} and more) of urea due to the induction of oxidative stress via the formation of ROS (Maleva et al., 2013, 2015). Our recent studies carried out on the Brazilian elodea (*E. densa*) showed that "the high dose of urea (5 mM) had a significant toxic effect on some physiological and biochemical characteristics such as the content of chlorophyll *a* and the activity of catalase, ascorbate and guaiacol peroxidases" (Maleva et al., 2016). However, the effect of urea on oxidative processes in other species of macrophytes is unknown. There are many studies about the influence of different pollutants (especially metals) on the development of pro- and antioxidant reactions in macrophyte leaves, but the data on the combined effect of metals and organic substances are rather scanty. Previously, in the model experiments (4 days of exposure of *E. densa*) we have found that adding urea (5 mM) to metals (100 μM Ni or Cu) could enhance their toxic effect in leaves, especially in the case of copper (Maleva et al., 2016). At the same time, it was shown that urea (1–4 g L^{-1}) could reduce the adverse effects of herbicides whereas the doubling of urea concentration (8 g L^{-1}) worsened this effect on *Perilla frutescens* (Zhang et al., 2014). Urea is often used as an effective nitrogen fertilizer in the world agriculture. Recently, it was observed that the application of different kinds of fertilizers not only increased the biomass of plants but could also enhance the absorption of metals from the substrate. The increase in Cd bioabsorption due to the application of urea fertilizer has been reported for two aquatic plants – *Veronica anagallis-aquatica* and *Epilobium laxum* in hydroponic experiments (Ahmad et al., 2016). It can be assumed that urea along with metals can act as a chelating agent and can form stable complexes between each other (Theophanides, 1987; Ibrahim, 2012). Furthermore, complexes of urea with some metals are used as fertilizers (Ibrahim, 2012).

However, there is no available study on macrophytes with collective action of urea and Cu/Ni that are essential elements for plant growth. Therefore our research is focused on comparative assessment of two submerged macrophytes *Egeria densa* and *Ceratophyllum demersum* on: a) metal accumulation ability namely nickel and copper during short-term exposure (48 h) and testing whether urea at moderate concentration (2 mM of urea, which close to the maximum permissible concentration of Urea for fishery water bodies (80 mg L^{-1} ~ 1.3 mM; Mironets et al., 1988)) affects this process; and b) the action of urea (separately and combined with nickel and copper treatment) on the content of photosynthetic pigments, total nitrogen, soluble protein, lipid peroxidation, urease activity and antioxidant reactions in the leaves of plants.

2. Materials and methods

2.1. Plant materials and growth conditions

Experiments were performed with two submerged plant species – *Egeria densa* (Planch.) Casp. (syn. *Elodea densa* or Brazilian elodea, Hydrocharitaceae family) and *Ceratophyllum demersum* L. (commonly known as hornwort, Ceratophyllaceae family). Both species having a cosmopolitan distribution in temperate and tropical regions in the world and very popular among aquarium plants as easily propagated by cuttings and undemanding in cultivation under laboratory conditions (Kumar and Prasad, 2004; Maleva et al., 2016). The root system of *E. densa* is not very strong, producing at intervals along the stem, whereas *C. demersum* does not form roots. Only the shoots of *E. densa* and *C. demersum* (15–20 cm in size, 20 samples per tank) previously raised in aquarium culture, incubated for 48 h in a nutrient solution containing 3.2 mg K L^{-1}; 1.9 mg N L^{-1}; 0.2 mg P L^{-1}; 0.02 mg Fe L^{-1}; 0.005 mg Mn L^{-1} at 23–25 °C under natural light (100–150 μM m^{-2} s^{-1}) and day: night period (10:14). Urea (2 mM); Ni (10 μM); Urea + Ni (2 mM + 10 μM); Cu (10 μM); Urea + Cu (2 mM + 10 μM) were mixed in the tanks (3.0 L), while plants without metal/urea treatment were used as a reference (control). Both Cu and Ni metal ions were mixed in sulfate form. The concentration of metals and urea was chosen by previously performed experimental previous tryouts (Maleva et al., 2013, 2015, 2016). The pH of the nutrient solution was measured before and after incubation by Waterproof Pocket pH tester (Hanna Instruments, Germany). As reported previously, the *Elodea* (*Egeria*) *densa*, as well as *Elodea canadensis* can alkalize the pH, especially in the presence of pollutants (Javed and Greger, 2001; Maleva et al., 2015). However, in case of *C. demersum*, the pH of the medium remained practically unchanged (Supplementary Table 1).

After the incubation, the shoots were completely washed with 0.01% Na_2EDTA followed by triple wash with double distilled deionized water. Fresh leaves were excised from the shoots and further saved for analysis. For the estimation of dry weight (DW), nickel and copper content, the leaves were dewatered on blotting paper, and one gram (in each replication) of fresh weight (FW) was dried in a hot air oven at 75 °C for 24 h.

2.2. Estimation of nickel and copper content

To analyze the Ni and Cu content in the leaves, the completely dried plant materials were crushed down in fine particles passed through a fine sieve and wet digested using 70% HNO_3 (AR grade). The metal concentrations were determined using Graphite furnace-atomic absorption spectrometry (GF-AAS, Analytik Jena, Vario 6, Germany) using flame: air-acetylene gas (Fuel flow for Cu: 50 L h^{-1}; for Ni: 55 L h^{-1}), burner: 100 mm, height – 5 mm, angle – 0 grd), nebulizer rate: 6.0 mL min^{-1}, wavelength (Cu: 324.8 nm, current – 3.0 mA, slit width – 1.2 nm; Ni: 232.0 nm, current – 6.0 mA, slit width – 0.2 nm) was used at a temperature between 1900 and 2300 °C. Double distilled deionized water (Milli-Q system, Millipore, USA) was used constantly for preparation of solution. JSC Ural Plant of Chemical Reagents, Russia: Ni (II): GSS 7265-96 and Cu(II): GSS 7255-95 were used for standard reference material (SRM). Calibration coefficients were ascertained ≥ 0.99. Less than 5% of relative variance was considered to retain the accuracy of the result. The recovery percentage of certified standards was 98.4% and 95.6% for Cu and Ni, respectively. Limit of detection was calculated as three times the standard deviation of the blank samples for each measured element and was 0.0001 and 0.0005 mg L^{-1} for Cu and Ni, respectively. The bioconcentration factor (BCF) was calculated as the ratio of the concentration of metal in leaves (mg/kg) to the concentration in medium (mg/L).

2.3. Measurement of photosynthetic pigment content

Chlorophylls and carotenoids (30 mg of fresh leaves) were extracted in dark with 5 mL of chilled absolute ethanol. Samples were centrifuged at 8,000 × *g* for 10 min and absorbance was recorded using Ultraviolet-visible spectrophotometer (PD303UV Apel, Japan) at 470, 649 and 664 nm wavelengths. The photosynthetic pigment content (mg g^{-1} dry weight) was measured according to the method described by Lichtenthaler (1987).