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Assessment of macro-micro element accumulation capabilities of *Elodea nuttallii* under gradient redox statuses with elevated NH_4-N concentrations

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Abstract – Aquatic plants often encounter various redox conditions in their natural environment. *Elodea nuttallii* (Planch.), a submerged aquatic macrophyte, has a flexile ability to use different nutrient sources from various environments. In the present study, *Elodea nuttallii* was subjected to various redox conditions (+400 mV to –180 mV) at both normal (2.5 ppm) and high (10 ppm) ammonium concentrations and evaluated for macro and micro element accumulation. A reduced environment was prepared by adding glucose to growth medium and nitrogen gas bubbling, while an oxic environment was executed by atmospheric air bubbling. Plants in oxygen-deprived conditions manifested heavy metal (HM) toxicity, such as reduction of biomass and photosynthetic pigments, excess generation of reactive oxygen species (ROS), lipid peroxidation and reduction of major macro elements. In reduced treatments, the bioaccumulation sequence for micro elements was Cu>Mn>Zn>Al>Cd>Fe>Pb at both normal and high NH_4-N concentrations. The combined effect of low redox state and high ammonium concentration had a strong physiological impact on the submerged macrophyte. However, macro- and micronutrient accumulation was more significantly affected by reduced environment than by a high NH_4-N concentration.

Keywords: anoxia, ammonium, *Elodea nuttallii,* macro-micro elements, accumulation, translocation

Introduction

Metal mobility and availability in sediments and in wetlands is governed by a number of sediment factors and processes; e.g. adsorption/desorption reactions, precipitation/dissolution and complexation/decomplexation, salinity, organic matter content, sulphur (S) and carbonate content, plant growth, pH and redox potential (E_H) as well as microorganism activity (DU LAING et al. 2009, MARÍA-CERVANTES et al. 2010). Oxidation and reduction processes subsequently affect pH (YU et al. 2007), which is directly related to stability and sol-

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ubility of various metals and nutrient elements in soil and sediment, and to their availability in plants (REDDY and PATRICK 1977). According to DEVAI and DELAUNE (1995), E_H of soil or sediment can range from –300 to + 700 mV and anaerobic soil or sediment exhibit redox potentials from + 350 mV to as low as –300 mV. Sediments/soil tend to undergo a series of sequential redox reactions in a homogenous environment when sediment redox status changes from aerobic (high E_H) to anaerobic (low E_H) conditions and vice versa. Major reactions, in order of decreasing E_H, are nitrification, denitrification, manganic manganese [Mn (IV)] reduction, ferric iron [Fe (III)] reduction, sulfate (SO_4^{2-}) reduction, and methanogenesis (PATRICK et al. 1996). In anoxic conditions, by reduction reactions, oxide elements such as phosphorus (P), molybdenum (Mo), cobalt (Co), copper (Cu), zinc (Zn) are often transformed to a more mobile and plant-available form (FRANCIS and DODGE 1990). Lower sediment pH under mildly oxic conditions increase the bioavailability of Al, Cu, Fe, Mn and Zn to rooted aquatic plants (JACKSON et al. 1993). Submerged aquatic plants adapt to detoxify reduced elements by releasing root oxygen to the rhizosphere, which is also governed by E_H condition and microbial oxygen demand (LASKOV et al. 2006). The concentration of metals in plants can be more than 100,000 times greater than in the associated water (ALBERS and CAMARDESE 1993). Recent studies on heavy metal (HM) toxicity revealed that these metals may cause molecular damage to plant cells either directly or indirectly through the excessive generation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\cdot}$) and superoxide radicals ($O_2^{\cdot -}$). These ROS can damage membranes and inactivate several enzymes by reacting very rapidly with DNA, lipids, pigments and proteins (WECKX and CLIJSTERS 1996). Thus, variation in redox conditions exerts a substantial influence on the physiological processes of plants.

Elodea nuttallii, a submerged aquatic rooted macrophyte, can absorb nutrients either by roots or shoots or by both together in varying proportions (BARKO et al. 1991). This species is well known as a hyper-accumulator of various metals and elements as well as being stress resistant to various environmental factors (MISHRA and TRIPATHI 2008). The capability of the shoots and roots of submerged macrophytes to accumulate trace metals allows their use in trace-element biomonitoring in lake ecosystems (BALDANTONI et al. 2004). Physical factors that fluctuate temporarily include pH, redox potential, temperature, salinity or light and in addition to the presence of other metal ions in the surrounding aquatic environment strongly affect metal uptake by submerged plants (FRITIOFF et al. 2005).

Sediment redox status and its effect in wetland plants and crops have been vigorously studied in the last three decades. The effect of a reduced environment on aquatic macrophytes is very slight (DELAUNE et al. 1999). Increased ammonium concentration and low redox status (reduced condition) in the natural habitat (due to pollution or eutropication) are two characteristics prominently associated with eutropic lakes, such as Plesne Lake in Central Europe (KOPÁÈEK et al. 2004). Furthermore, in a reduced environment different oxidized elements become available in the surrounding environment. Trace elements like Cu, Fe, Mn and Zn are essential minerals for normal growth of aquatic macrophytes but excessive concentration might have a deleterious effect by disordering physiological and biochemical processes in the cells. These elements give especially grounds for concern as they are not biodegradable (LU et al. 2007) and contribute to the food chain. The aim of the present work was to assess the significance of reducing conditions on (i) the release of inorganic contaminants, (ii) their concentration and translocation, and (iii) the oxidative damage caused by these elements under normal and high NH_4-N concentrations in *Elodea nuttallii*.

Materials and methods

Sediment and plant collection

The sediment was collected from a pond in Oaso Park near Tokyo, in December, 2010. The organic-rich sediment (organic matter content >5%) was derived from the top surface (<15 cm depth) of pond sediment. *Elodea nuttallii* (Planch.) was collected from Moto-Arakawa River, Saitama, Japan, in April, 2011. Collected plants were allowed to adapt to laboratory conditions for 2 weeks in the experimental tanks, where the temperature was maintained at 25 °C, with a relative air humidity of 90% and a photon flux density of approximately 100 μmol m^{-2} s^{-1} was provided by fluorescent lamp in a 12h light/12h dark cycle.

Experimental set-up

Elodea nuttallii was subjected to gradient redox potentials under normal and high NH_4-N concentrations. Since it was difficult to keep a constant redox potential throughout the experiment period, a range of potentials was maintained. Three levels of redox potential were used, as (i) +400 mV ~ +440 mV, (Oxic; O_1), (ii) –5 mV ~ +5 mV (hypoxic/moderately reduced; O_2) and (iii) –180 mV ~ –120 mV (anoxic/highly reduced; O_3) (Fig. 1). In the case of a nitrogen source, the suitable NH_4-N concentration for the plant is 2.5 ppm (OZIMEK et al. 1993). Here, two different NH_4-N concentrations [2.5 (N_1) and 10 (N_2) ppm] were used (Fig. 1). The experiment was conducted in microcosms (MCs), each consisting of a 6 L (15.7 × 15.7 × 24.5 cm^3) glass vessel which was hermetically sealed with an air-tight lid. Each MC was filled with 600 g of air-dried sediment and deionized water in a 1:5 ratio. Then, growth medium contained 5% Hoagland nutrient solution (HOAGLAND and ARNON 1950) was mixed, and ammonium sulfate was added to adjust the required NH_4-N concentration.

Highly reduced and moderately reduced microcosms were prepared following the method developed by YU et al. (2007). Glucose, a simple carbon source, was used in this experiment during the 22-day incubation period. At the beginning of incubation, 8.16 g glucose was added to the reduced (MC 3) and highly reduced microcosms (MC 4) on days 1 and 3, and twice that amount was added on day 5. On day 14, again, 8.16 g glucose was added to MC 4. Continuous flushing of N_2 gas was carried out for the last 3 days for a

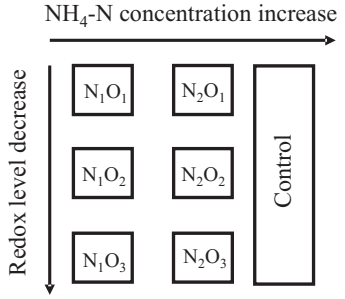

Fig. 1. Layout of the experimental set-up (seven microcosms per treatment). NH_4-N Concentration and redox level are presented as N and O, respectively. Microcosms were randomly distributed with equal spacing in growth chamber.

hypoxic/moderately reduced (MC3) condition and for the last 7 days for anoxic/highly reduced (MC4) condition to reduce the redox potential (E_H) values to approximately 5 mV and 180 mV, respectively. For oxic treatments, continuous bubbling with atmospheric air was used. E_H and pH were measured four times a day using four portable pH/ORP meters (POT-101 M, SIBATA, Japan). For control, 5% Hoagland nutrient medium was used without any further treatment. The temperature was maintained at 23 ± 2 °C in a room with fluorescent lighting. No attempt was made to control the pH of the sediment suspensions. After the incubation period (22 days), eight plants (approximately 12–14 cm in height) were planted in each experimental tank. Then, the experiment was continued for 14 days, with continued N_2 gas flushing to maintain the required E_H potential. In total, three treatments, each with 7 microcosms, were applied.

Sediment, plant and water analysis

Sediment samples were air-dried, homogenized and sieved to < 2 mm. The particle sizes of the sediment samples (in terms of D50) were determined using sieves according to the American Society for Testing and Materials protocol (ASTM D422-63, 2002). Plants were carefully washed using tap water and finally with distilled water, and were separated into leaves, shoots and roots. Plant materials were dried using an oven drier at 60 °C until constant weight. Plant materials were reweighed (for dry weight) and homogenized by grinding into fine powder using a mortar and pestle. Powdered samples were stored in airtight vials for subsequent analysis. Total nitrogen (TN) and total carbon (TC) of powdered plant samples were measured by CHN coder (YANACO MT-3). About 10 mg of dried plant sample and 200 mg of dried sediment sample were digested at 200 °C with di-acid mixture (nitric acid : perchloric acid; 1:2) until evolution of nitrous gas was stopped and the digest became clear. The digests were diluted with distilled water to a total of 100 mL and passed through Whatman 42 filter paper. Organic matters in the sediment were measured by the WALKLEY and BLACK (1934) method. The concentrations of the following elements were measured in the sediment and in the plant samples: Fe, Mn, Zn, Pb, Ca, Mg, Cu and K with atomic absorption spectrophotometer (AAS; Shimadzu AA-660 G) using the direct air-acetylene flame method, and the concentration of Al and Cd were determined with a graphite furnace atomizer (GFA-4B), according to the instructions and procedure. Total phosphorus (TP) and total sulphur (TS) were measured using the ascorbic acid method and the barium chloride method respectively. Replicate samples were analyzed separately, analyses were done in duplicate, and results for plant materials and sediments were calculated on a dry weight basis. Water samples were collected at 7 day intervals and were passed through Whatman glass microfibre filters GF/C and stored at 4 °C until analysis. The concentrations of Fe, Mn, Zn, Pb, Ca, Mg, Cu, K, Al, Cd and TS of water sample were measured following the methods used for sediment and plant sample analysis. Ammonium nitrogen was determined by autoanalyzer (Technicon II TRAAC 800).

Biomass increment

On the14th day after treatment (DAT), two plants from each tank were harvested, and cleaned with tap water, and fresh biomass was measured after blotting with laboratory towel. The fresh biomass increment was calculated as the percent increment of plant mass relative to initial fresh mass at the time of transplanting, using the following equation:

Where *Bt* is the increased biomass (% relative to initial fresh biomass) at the 14th DAT, *Ft* is the fresh biomass at 14th DAT and *Fo* is the initial fresh biomass of the plant.

Chlorophyll content, carotenoid content and chlorophyll flurorescence

Photosynthetic pigments were extracted in 95% ethanol in the dark for 24 h. Afterwards, the sample was centrifuged for 10 min at 8000 × g. Finally, supernatants were read at 665 and 649 nm for chlorophyll *a* and chlorophyll *b*, respectively, and at 470 nm for carotenoid content using spectrophotometer (Shimadzu UV-1700, Japan). The contents of chlorophylls and carotenoid were calculated according to LICHTENTHALER (1987). Chlorophyll *a* fluorescence measurements were performed with a handy flurocam (FC 1000-H, Photon Systems Instruments, Czech Republic) using auto image segmentation. Maximum photochemical efficiency of PSII (Fv/Fm), the activity of PSII (Fv/Fo) and electron transport rate (ETR) through PSII (Fm/Fo) were determined and used as a stress indicator for plants.

H_2O_2 concentration and peroxidase activity

Endogenous H_2O_2 concentrations were analyzed following the method of CERVILLA et al. (2007), where samples were extracted with cold acetone. Phosphate buffer (0.1 mol L^{-1}) at pH 6 was used to make extracts suitable for peroxidase (POD) activity measurements. POD was determined according to the method described by GOEL et al. (2003).

Lipid peroxidation and proline concentration

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA), a product of lipid peroxidation in the plant samples estimated by thiobarbituric acid (TBA) reaction (HEATH and PACKER 1968). The concentration of proline was measured with the BATES et al. (1973) method. Plant material was homogenized with 10 mL of 3% (v/v) sulfosalicylic acid. Free proline present in the supernatant was treated with acid-ninhydrin at 80 °C for 1 h and measured spectrophotometrically at 520 nm.

Bioconcentration factor and translocation factor

The bioconcentration/bioaccumulation factor (BCF) is an index to express the ability of a plant to accumulate metal with respect to metal concentration in substrate. BCF (for whole plant) was calculated by the following formula:

The translocation factor (TF) is an indication of the ability of the plant to translocate metals from the roots to the aerial parts of the plant. TF was calculated by the following formula:

Translocation factors (TF) for trace elements between sediment and roots and within a plant were expressed by the ratios of [Trace element] $_{sediment}$/ [Trace element] $_{root}$ and [Trace eleament] $_{root}$/ [Trace element] $_{(shoot + leaves)}$ to show trace elements translocation properties from sediment to roots and roots to shoots, respectively.

Statistical analysis

The experimental set up was a completely randomized design, and average values of three treatments were considered. Data were analyzed statistically using the SPSS 13.0 software package, by ANOVA and by Tukey's multiple range tests to determine differences between means. Before performing a statistical analysis, data were checked for normal distribution. Pearson correlation analysis was carried out to explore the correlations.

Results

Biomass increment

Plants subjected to a high concentration of NH_4-N (10 ppm) along with hypoxic/anoxic treatments showed brown-black discoloration of the leaves and biomass increment values were negative. Increment of ammonium even in oxic treatment considerably reduced biomass (Fig. 2). When oxygen level decreased, biomass was more affected at both ammonium levels. At 2.5 ppm, NH_4-N nutrition condition by hypoxic and anoxic treatment, the fresh biomass declined by 73.02 and 80%, respectively.

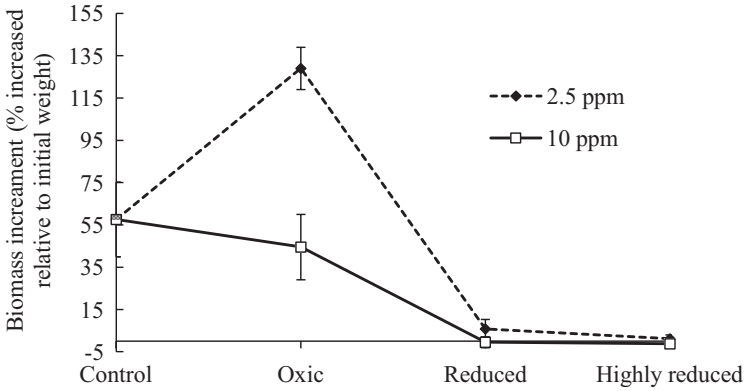

Fig. 2. Effect of NH4-N concentrations under various redox conditions on biomass of *Elodea nuttallii*. The data are presented as the mean ± SD.

Photosynthetic pigments and chlorophyll fluorescence

Photosynthetic pigments including Chl *a*, Chl *b* and carotenoid content showed a slight falling trend with the increment of NH_4-N concentration in oxic treatments (Tab. 1). At hypoxic and anoxic treatments both chlorophyll and carotenoid levels significantly declined even when NH_4-N concentration was at a normal level (2.5 ppm), suggesting that hypoxia itself was sufficient to affect both chlorophyll and carotenoid content. Moreover, carotenoid seemed to be affected more severely and found absent at high reduced treatment at 10 NH_4-N concentration.

Maximum photochemical efficiency of PSII (Fv/Fm), the activity of PSII (Fv/F_0) and electron transport rate (ETR) through PSII (Fm/F_0) are presented in table 1. Their values were not significantly affected by high NH_4-N concentration in oxic treatment but were

significantly decreased in plants grown in hypoxic and anoxic treatments. Increment of NH_4-N concentration in hypoxic and anoxic treatments more significantly affected their values (Tab. 1).

Tab. 1. Photosynthetic pigments and chlorophyll fluorescence parameters (Mean ± SD) of *Elodea nuttallii* under different conditions (NH_4-N concentrations) and treatments (oxic to highly reduce).

Parameters	Control	2.5 ppm NH_4-N			10 ppm NH_4-N		
		Oxic	Reduced	Highly reduced	Oxic	Reduced	Highly reduced
Chl*a*	2.9 ± 0.1	3.2 ± 0.2	1.7 ± 0.2*	1.4 ± 0.2**	2.7 ± 0.1	1.2 ± 0.1**	1.0 ± 0.0***
Chl*b*	1.4 ± 0.1	1.7 ± 0.2	1.1 ± 0.2*	1.0 ± 0.0**	1.3 ± 0.2	1.0 ± 0.1**	0.9 ± 0.1**
Carotenoid	1.1 ± 0.0	1.3 ± 0.2	0.9 ± 0.0*	0.5 ± 0.1**	0.9 ± 0.1*	0.3 ± 0.0**	0.0 ± 0.0***
Fv/Fm	0.7 ± 0.0	0.8 ± 0.0	0.6 ± 0.0	0.5 ± 0.0*	0.7 ± 0.0	0.5 ± 0.0*	0.4 ± 0.0*
Fv/F_0	4.3 ± 0.1	4.7 ± 0.1	2.5 ± 0.2**	1.8 ± 0.2**	3.9 ± 0.3*	2.0 ± 0.0**	1.3 ± 0.2***
Fm/ F_0	5.6 ± 0.2	5.9 ± 0.1	3.7 ± 0.1**	3.1 ± 0.3***	5.2 ± 0.2	3.4 ± 0.2**	2.9 ± 0.2***

Significance at: $p < 0.05$*; $p < 0.01$**; $p < 0.001$***.

Lipid peroxidation rate and proline content

Lipid peroxidation rate was determined by measuring MDA content. This parameter was significantly increased (Fig. 3a) in plants that were exposed to reduced treatments ($P < 0.01$). Moreover, increment of NH_4-N concentration in reduced treatments accelerated the increment of MDA level, and hence, maximum MDA content was observed in plant under highly reduced treatment at 10 ppm NH_4-N concentration.

Proline level declined slightly in plants under oxic treatments with high NH_4-N concentrations (Fig. 3b), suggesting high ammonium concentration has a weak effect on the proline content of the plant. In reduced and highly reduced treatments, the proline content was considerably reduced (Fig. 3b). Plants in oxic treatment with 2.5 ppm NH_4-N concentration showed the highest proline content (1.21 mg g^{-1} FW), whereas plants in anoxic treatment with 10 ppm NH_4-N concentration showed the lowest proline content (0.22 mg g^{-1} FW).

Endogenous H_2O_2 generation and POD activities

A significantly higher H_2O_2 concentration ($p < 0.05$) was found throughout the experimental period in reduced and highly reduced treatments (Figs. 4a). Similar up-regulation was also observed for POD activity (Fig. 4b). In oxic treatments, increment of NH_4-N concentration exhibited a slight increasing trend in both H_2O_2 level and POD activity (Figs. 4a, 4b). H_2O_2 concentration and POD activity were positively correlated in all treatments and conditions (oxic, $r = 0.847$, $n = 16$, $p < 0.01$; reduced, $r = 0.948$, $n = 16$, $p < 0.001$ and highly reduced $r = 0.929$, $n = 16$, $p < 0.001$).

Element bioaccumulation and translocation in plant

BCF and TF were calculated to study the accumulation characteristics of different essential and non-essential elements in different body parts (leaf, shoot and root) of the plant. Sig-

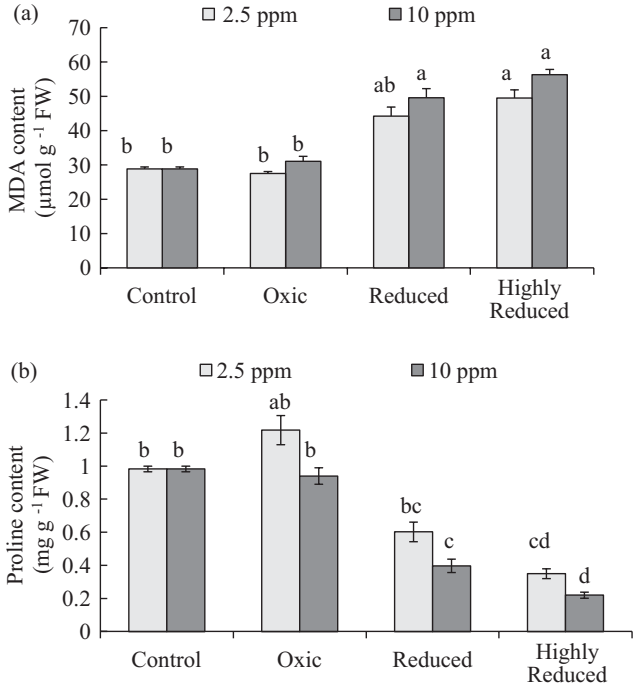

Fig. 3. Effects of NH4-N concentrations under various redox conditions on MDA content and proline concentration of *Elodea nuttallii*; (a) MDA content and (b) proline concentration. The data are presented as the mean ± SD. One-way ANOVA followed by Tukey's test was used to determine the significance of difference between treatments ($p < 0.05$).

Tab. 2. Bioaccumulation factor of elements in *Elodea nuttallii* (Mean ± SD) under different conditions (NH_4-N concentrations) and treatments (oxic to highly reduce).

Elements	Control	2.5 ppm			10.0 ppm		
		Oxic	Reduced	Highly reduced	Oxic	Reduced	Highly reduced
Ca	3.6 ± 0.6^{cd}	3.3 ± 0.3^{cd}	0.4 ± 0.1^{e}	0.2 ± 0.1^{ef}	2.9 ± 0.2^{cd}	0.3 ± 0.1^{e}	0.3 ± 0.1^{e}
Mg	9.6 ± 3.3^{b}	10.9 ± 3.8^{b}	4.9 ± 1.1^{c}	3.8 ± 0.8^{c}	10.1 ± 3.1^{b}	4.2 ± 0.5^{c}	3.1 ± 0.2^{cd}
K	14.3 ± 4.6^{a}	13.9 ± 4.1^{a}	4.9 ± 1.6^{c}	4.2 ± 1.1^{c}	12.6 ± 4.8^{a}	4.2 ± 1.0^{c}	3.6 ± 0.8^{cd}
S	2.3 ± 1.1^{c}	2.6 ± 0.8^{c}	3.9 ± 1.2^{bc}	4.0 ± 1.2^{bc}	2.7 ± 1.0^{c}	3.3 ± 0.8^{c}	3.5 ± 0.6^{c}
Cu	0.6 ± 0.1^{cd}	0.7 ± 0.2^{cd}	4.6 ± 0.5^{ac}	5.9 ± 0.6^{ac}	0.7 ± 0.2^{cd}	5.5 ± 0.4^{ac}	6.3 ± 0.8^{ad}
Mn	0.5 ± 0.2^{c}	0.5 ± 0.1^{c}	2.8 ± 0.2^{b}	2.9 ± 0.4^{b}	0.5 ± 0.1^{c}	3.4 ± 0.1^{a}	3.1 ± 0.0^{ab}
Zn	0.8 ± 0.1^{de}	0.9 ± 0.1^{de}	1.1 ± 0.1^{de}	1.2 ± 0.0^{cd}	0.9 ± 0.1^{de}	1.3 ± 0.1^{cd}	1.5 ± 0.2^{c}
Fe	0.3 ± 0.0^{c}	0.3 ± 0.0^{c}	0.7 ± 0.1^{b}	0.8 ± 0.1^{b}	0.3 ± 0.0^{c}	0.9 ± 0.1^{b}	1.0 ± 0.0^{a}
Al	0.7 ± 0.2^{de}	0.7 ± 0.1^{de}	1.1 ± 0.2^{cd}	1.1 ± 0.2^{cd}	0.7 ± 0.1^{de}	1.1 ± 0.1^{cd}	1.1 ± 0.1^{cd}
Pb	0.1 ± 0.0^{e}	0.1 ± 0.0^{e}	0.5 ± 0.1^{c}	0.6 ± 0.0^{c}	0.1 ± 0.0^{e}	0.6 ± 0.1^{c}	0.6 ± 0.0^{c}
Cd	0.1 ± 0.0^{e}	0.0 ± 0.0^{e}	0.9 ± 0.1^{c}	1.1 ± 0.1^{b}	0.0 ± 0.0^{e}	0.8 ± 0.1^{c}	0.9 ± 0.2^{c}

Different letter superscripts indicate significant differences between treatments, and same superscript letter as control indicate no significant difference.

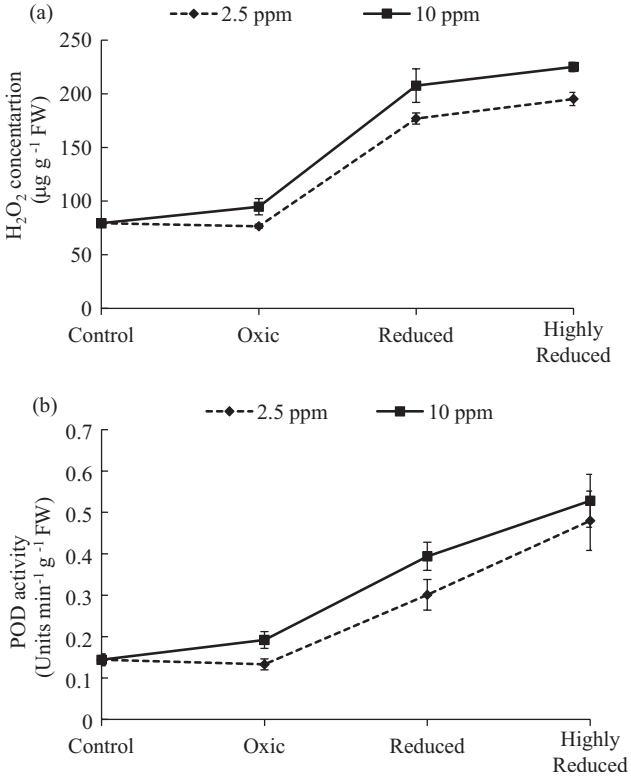

Fig. 4. Variations in H_2O_2 concentration and POD activity of *Elodea nuttallii,* grown at different NH4-N concentrations under various redox statuses; (a) H_2O_2 concentration and (b) POD activity. The data are presented as the mean ± SD.

nificant BCF differences ($p < 0.001$) were found between different redox treatments at both NH_4-N conditions for both macro and micro elements (Tab. 2). In both NH_4-N concentrations with hypoxia/anoxia, BCF was downregulated for Ca, Mg and K, but upregulated for S, Fe, Cu, Mn, Zn, Cd, Pb and Al. In an oxic treatment, the highest bioaccumulation was found for K, but in a highly reduced treatment Cu showed the highest value.

Translocation of elements from sediment to roots seems more significantly affected by redox treatments than by NH_4-N conditions (Tab. 3). In reduced (hypoxic and anoxic) treatments, the translocation factor from sediment to root was increased for Ca, Mg and K, whereas, downregulated TF was observed for Fe, Cu, Mn, Cd, Pb and Al (Tab. 3). Moreover, in a highly reduced treatment, the TF of Ca was mostly increased, while the TF of Cd mostly declined (Tab. 3). However, translocations of S and Zn were not significantly affected by redox treatments and NH_4-N conditions (Tab. 3). On the other hand, translocation of elements from roots to shoot and leaf was not affected by NH_4-N conditions but was affected by redox treatments (Tab. 4). Translocation of Ca, Fe, Cu and Mn was decreased by reduced treatment; however, translocation of Cd was decreased in oxic treatment under both NH_4-N conditions (Tab. 4).

Tab. 3. Translocation factor (sediment/root) of elements in *Elodea nuttallii* under different conditions (NH_4-N concentrations) and treatments (oxic to highly reduce).

Elements	Control	2.5 ppm			10.0 ppm		
		Oxic	Reduced	Highly reduced	Oxic	Reduced	Highly reduced
Ca	2.9 ± 0.9^{d}	3.0 ± 0.8^{d}	13.6 ± 9.0^{b}	30.2 ± 3.0^{a}	3.0 ± 0.6^{d}	13.6 ± 9.0^{b}	39.2 ± 9.8^{a}
Mg	0.5 ± 0.2^{d}	0.6 ± 0.1^{d}	1.4 ± 0.2^{c}	1.4 ± 0.2^{c}	0.6 ± 0.1^{d}	1.6 ± 0.3^{b}	1.6 ± 0.1^{b}
K	0.3 ± 0.0^{d}	0.3 ± 0.0^{d}	0.9 ± 0.2^{c}	0.9 ± 0.1^{c}	0.3 ± 0.0^{d}	0.9 ± 0.0^{c}	1.0 ± 0.2^{c}
S	1.4 ± 0.5^{c}	1.1 ± 0.1^{c}	1.0 ± 0.2^{d}	1.0 ± 0.2^{d}	1.2 ± 0.3^{c}	1.2 ± 0.3^{c}	1.3 ± 0.2^{c}
Fe	9.3 ± 1.6^{c}	7.9 ± 0.9^{c}	6.9 ± 0.2^{d}	6.6 ± 0.1^{d}	7.0 ± 0.0^{d}	5.8 ± 1.3^{de}	5.9 ± 2.1^{de}
Cu	4.8 ± 0.9^{b}	3.2 ± 0.4^{c}	1.4 ± 0.3^{cd}	1.2 ± 0.1^{cd}	3.9 ± 1.4^{bc}	1.3 ± 0.3^{cd}	1.4 ± 0.4^{cd}
Mn	4.9 ± 1.7^{b}	4.9 ± 0.3^{b}	1.2 ± 0.1^{c}	1.1 ± 0.1^{c}	4.6 ± 0.4^{b}	1.2 ± 0.1^{c}	1.0 ± 0.1^{cd}
Zn	0.7 ± 0.0^{a}	0.7 ± 0.0^{a}	0.6 ± 0.0^{a}	0.8 ± 0.1^{a}	0.7 ± 0.0^{a}	0.7 ± 0.1^{a}	0.8 ± 0.1^{a}
Cd	18.6 ± 7.6^{b}	23.8 ± 4.2^{ab}	1.9 ± 0.7^{d}	1.5 ± 0.3^{d}	28.7 ± 5.2^{a}	1.9 ± 0.4^{d}	1.7 ± 0.1^{d}
Pb	34.2 ± 4.0^{b}	32.2 ± 5.7^{b}	24.2 ± 2.4^{ab}	22.4 ± 2.3^{ab}	31.9 ± 6.1^{b}	19.8 ± 1.1^{a}	23.5 ± 2.0^{ab}
Al	4.0 ± 0.0^{c}	4.1 ± 0.1^{c}	2.8 ± 0.0^{cd}	2.8 ± 0.0^{cd}	4.1 ± 0.0^{c}	3.0 ± 0.0^{cd}	2.9 ± 0.0^{cd}

Different letter superscripts indicate significant differences between treatments, and same superscript letter as control indicate no significant difference.

Tab. 4. Translocation factor (root/(shoot+leaf) of elements in *Elodea nuttallii* under different conditions (NH_4-N concentrations) and treatments (oxic to highly reduce).

Elements	Control	2.5 ppm			10 ppm		
		Oxic	Reduced	Highly reduced	Oxic	Reduced	Highly reduced
Ca	0.5 ± 0.1^{c}	0.5 ± 0.1^{c}	0.3 ± 0.1^{cd}	0.2 ± 0.0^{d}	0.5 ± 0.0^{c}	0.3 ± 0.1^{cd}	0.1 ± 0.0^{d}
Mg	0.3 ± 0.1^{c}	0.2 ± 0.0^{cd}	0.2 ± 0.0^{cd}	0.2 ± 0.0^{cd}	0.2 ± 0.0^{cd}	0.2 ± 0.0^{cd}	0.3 ± 0.0^{c}
K	0.4 ± 0.1^{c}	0.4 ± 0.1^{c}	0.3 ± 0.0^{cd}	0.3 ± 0.1^{cd}	0.3 ± 0.0^{cd}	0.3 ± 0.0^{cd}	0.3 ± 0.1^{cd}
S	0.5 ± 0.3^{c}	0.5 ± 0.2^{c}	0.4 ± 0.1^{c}	0.3 ± 0.0^{cd}	0.5 ± 0.2^{c}	0.3 ± 0.1^{cd}	0.3 ± 0.0^{cd}
Fe	0.7 ± 0.2^{c}	0.6 ± 0.0^{c}	0.3 ± 0.1^{d}	0.3 ± 0.0^{d}	0.7 ± 0.1^{c}	0.3 ± 0.0^{d}	0.2 ± 0.0^{d}
Cu	0.5 ± 0.1^{c}	0.7 ± 0.2^{bc}	0.2 ± 0.1^{d}	0.2 ± 0.0^{d}	0.6 ± 0.1^{c}	0.2 ± 0.0^{d}	0.1 ± 0.0^{de}
Mn	0.8 ± 0.2^{b}	0.6 ± 0.0^{bc}	0.4 ± 0.0^{c}	0.4 ± 0.0^{c}	0.6 ± 0.0^{bc}	0.4 ± 0.0^{c}	0.4 ± 0.0^{c}
Zn	0.3 ± 0.0^{d}	0.5 ± 0.0^{cd}	0.4 ± 0.0^{d}	0.3 ± 0.1^{de}	0.5 ± 0.0^{cd}	0.3 ± 0.0^{d}	0.3 ± 0.0^{d}
Cd	1.8 ± 1.0^{c}	0.9 ± 0.5^{d}	2.3 ± 0.8^{bc}	1.9 ± 0.2^{c}	0.8 ± 0.3^{d}	1.9 ± 0.3^{c}	1.7 ± 0.0^{c}
Pb	0.3 ± 0.0^{b}	0.4 ± 0.1^{ab}	0.1 ± 0.0^{d}	0.1 ± 0.0^{d}	0.4 ± 0.1^{ab}	0.1 ± 0.0^{d}	0.1 ± 0.0^{d}
Al	0.5 ± 0.0^{c}	0.5 ± 0.0^{c}	0.4 ± 0.0^{cd}	0.4 ± 0.0^{cd}	0.5 ± 0.0^{c}	0.4 ± 0.0^{cd}	0.4 ± 0.0^{cd}

Different letter superscripts indicate significant differences between treatments, and same superscript letter as control indicate no significant difference.

Discussion

Reducing sediment conditions comprehend sediment oxygen deprivation, at the same time producing various compounds in sediment, many of which are considered highly phytotoxic (PEZESHKI and DELAUNE 2012). However, concentration of elements as well as

their speciation (physiochemical form and associations with sediment constituents) also affects their mobility and toxicity (KABATA-PENDIAS and PENDIAS 1992). The results of the present study revealed the combined effects of low redox condition and high ammonium concentration on macro-micro nutrient accumulation in the plant. Fe, Mn, Cu, Zn, Cd, Pb and Al were soluble at low pH, and alteration in redox conditions affects their speciation as well as solubility (TAKENO 2005, DU LAING et al. 2009, MILLER et al. 2010). In oxic treatments, low concentrations of Fe and Mn were found, which might be the result of the formation of Fe and Mn (hydrate) oxides at high E_H (YU et al. 2007), and these oxides are very slightly soluble (GAMBRELL 1994).

The phyotoxicity due to different elements depends on metal type, metal concentration and duration of exposure (ODJEGBA and FASIDI 2007). The metal uptake and distribution in submerged plant species vary according to the relative concentration of the elements in the environment, the growth of the plant, type of absorption mechanism, metal speciation, metal stability and constants with ligands, redox potential and pH at water-sediment interface, light, and microbial activity (NAGAJYOTI et al. 2010). According to MARKERT and WTOROVA (1992), the presence of a high concentration of heavy metals (micro elements or trace metals) seems to be directly associated with the exclusion of nutritional elements. In our study, plants in reduced treatment were observed for exclusion phenomenon for macro elements (K, Ca and Mg), and consequently, their concentration declined below critical level in the plant (Tab. 2), and increased in water sample (Tab. 5). Among K, Ca and Mg, the most significant decrease was observed in Ca (0.3 ppm) (Tab. 2). The BCF sequence for bioaacumulated micro elements was Cu>Mn>Zn>Al>Cd>Fe>Pb in both NH_4-N conditions under reduced treatments (Tab. 2). Trace metal concentrations in aquatic plants vary considerably according to the part of the plant as well as chemical characteristics of the elements. BALDANTONI et al. (2004) concluded that a submerged macrophyte takes up the elements in

Tab. 5. Concentration (μg L^{-1}, mean ± SD) of elements in water of experimental tank under different conditions (NH_4-N concentrations) and treatments (oxic to highly reduce).

Elements	Control	2.5 ppm NH_4-N			10 ppm NH_4-N		
		Oxic	Reduced	Highly reduced	Oxic	Reduced	Highly reduced
Ca	11.3 ± 1.1^{c}	10.6 ± 2.8^{c}	16.6 ± 3.2^{b}	18.7 ± 1.9^{a}	11.9 ± 2.8^{c}	17.6 ± 3.8^{b}	20.4 ± 2.6^{a}
Mg	9.1 ± 1.3^{c}	10.7 ± 1.1^{c}	12.9 ± 1.7^{ab}	15.2 ± 1.5^{a}	11.1 ± 1.0^{c}	13.3 ± 1.4^{b}	17.0 ± 0.8^{a}
K	12.7 ± 2.1^{d}	12.6 ± 1.6^{c}	19.7 ± 3.4^{b}	23.3 ± 1.7^{a}	12.5 ± 2.3^{c}	21.0 ± 3.0^{b}	25.5 ± 4.1^{a}
S	15.7 ± 3.2^{d}	15.4 ± 1.8^{d}	19.2 ± 2.0^{cd}	22.1 ± 3.9^{b}	16.3 ± 3.2^{d}	23.8 ± 2.2^{b}	28.7 ± 3.6^{a}
Cu	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.9 ± 0.2^{a}	1.3 ± 0.3^{a}	0.0 ± 0.0^{b}	1.1 ± 0.2^{a}	1.4 ± 0.3^{a}
Mn	1.3 ± 0.1^{c}	1.2 ± 0.2^{c}	7.2 ± 0.7^{b}	10.0 ± 1.4^{a}	1.4 ± 0.1^{c}	9.1 ± 2.4^{b}	14.6 ± 0.9^{a}
Zn	2.5 ± 0.4^{c}	2.6 ± 0.3^{c}	5.2 ± 1.5^{b}	8.4 ± 1.9^{a}	2.7 ± 0.3^{b}	10.7 ± 1.1^{a}	11.8 ± 1.2^{a}
Fe	4.1 ± 1.7^{d}	4.4 ± 1.2^{d}	41.4 ± 3.7^{a}	57.8 ± 7.2^{a}	4.6 ± 2.1^{d}	50.9 ± 5.4^{a}	72.7 ± 8.2^{a}
Al	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	3.2 ± 0.1^{b}	4.3 ± 0.7^{a}	0.0 ± 0.0^{c}	4.0 ± 0.2^{b}	5.1 ± 0.5^{a}
Pb	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.2 ± 0.0^{a}	0.3 ± 0.0^{a}	0.0 ± 0.0^{b}	0.3 ± 0.0^{a}	0.4 ± 0.0^{a}
Cd(μg/L)	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.1 ± 0.0^{a}	0.2 ± 0.0^{a}	0.0 ± 0.0^{b}	0.2 ± 0.0^{a}	0.3 ± 0.0^{a}

Different letter superscripts indicate significant differences between treatments, and same superscript letter as control indicate no significant difference.

the shoots from water by the roots. In reduced treatments, trace metal contents of water sample have also been increased, which is probably due to E_H and pH effects, because at low E_H and pH such elements were solubilized in water from sediment (Tabs. 5, 6). Plant uptake of metal is mainly dependent on metal mobility and availability in sediment. Uptake of different metals also depends on protein transporters (FOULKES 2000). Pb, Zn, Cd, Fe, Cu are taken up at the cell surface through the cation channel (WELCH and NORVELL 1999, COSIO et al. 2004), so they compete with each other and exclude Ca ion. In our experiment, inreduced treatments, Cu^{+2} was more considerably bioaccumulated than Fe^{+2}, which might be due to Fe^{+2} and Cu^{+2} competing with each other for binding sites on the cell wall and being taken into the cell walls of plants (FOX and GUERINOT 1998). The accumulation of Zn by the plant was also low though this element was bioavailable in the surrounding environment. The uptake of metals was also found to be pH dependent (WANG et al. 2006) although in certain cases no pH effect was seen. The elements Al and Pb were found to be less accumulated in plants, which might be due to the above reason. In reduced treatments, metal accumulation in shoot and leaf was found to be higher than that in root, which might be due to the direct uptake by the shoot and leaves or from root to shoot by acropetal transport. Since roots degenerate and are greatly reduced in size due to metal toxicity (BASIOUNY et al. 1977), their potential for metal uptake might be limited.

Tab. 6. Redox potential and pH (mean ± SD) values of growth medium under different conditions (NH_4-N concentrations) and treatments (oxic to highly reduce).

Parameters	Control	2.5 ppm NH_4-N			10 ppm NH_4-N		
		Oxic	Reduced	Highly reduced	Oxic	Reduced	Highly reduced
E_H	288 ± 16.4^{b}	440 ± 11.3^{a}	-4 ± 1.1^{d}	-150 ± 17.4^{f}	432 ± 14.1^{a}	-2 ± 3.8^{d}	-157 ± 18.3^{f}
pH	6.9 ± 0.5^{b}	7.4 ± 1.0^{ab}	4.5 ± 0.3^{c}	4.1 ± 0.1^{c}	7.1 ± 0.8^{ab}	4.2 ± 0.4^{c}	4.1 ± 0.6^{c}

Different letter superscripts indicate significant differences between treatments, and same superscript letter as control indicate no significant difference.

Heavy metals could lead to oxidative damage to aquatic plants through ROS generation (MITTLER 2002). This was particularly crucial for photosynthetic organisms which generate ROS constantly during normal photosynthesis. Chlorophyll concentration was higher in plants in oxic treatments with a normal NH_4-N concentration (2.5 ppm), whereas at higher NH_4-N concentrations, the chlorophyll level declined significantly. Conversely, low redox potential affects chlorophyll synthesis at normal to high NH_4-N concentrations. The loss of chlorophyll contents consequently disrupts the photosynthetic machinery, thus the electron transport rates of PSI and PSII are disturbed, which leads to the generation of ROS. In the present study, decrease of chlorophyll content was probably achieved both by reaction with biosynthetic enzymes as well as peroxidase mediated degradation (ASADA 1994). In addition, carotenoid represents the other group of photosynthetic pigments that are highly effective in quenching chlorophyll triplet states and singlet oxygen (LICHTENTHALER 1987). The degree of anoxia damage to the photosynthetic apparatus in different oxygen-deprived conditions was determined by chlorophyll fluorescence of PSII in dark-adapted leaf, where the Fv/Fm values were decreased with increased oxygen deprivation (<0.4). Pronounced fluorescence decay in plants was observed under reduced environments, which might be due to

the substitution of Mg by other metals (such as Cu, Pb, Cd). The Fv/Fm ratio, the maximum quantum yield of PSII photochemistry, is frequently used as an indicator of photoinhibition or of other kinds of stress to photosystem II (CALATAYUD and BARRENO 2004).

Membrane lipids and proteins are especially prone to attack by free radicals. Proline accumulation is considered to be involved in stress resistance mechanisms (LUTTS et al. 1999). Decrease in proline content in the plant under reduced treatments might be due to the dysfunction of sulphydryl groups during heavy metal transportation into the plants (NAGOOR 1999), which affects protein synthesis. The increased activity of protease or other catabolic enzymes that are activated by heavy metals might be another reason (GUPTA et al. 1996). The results of the present study indicate that plants under reduced treatments seemed to be more vulnerable to metal toxicity as more than one metal was present at a toxic level in reduced treatments. Lipid peroxidation profoundly alters the structure of membranes and modifies their enzymatic and transport activities (RAI 1995). Increased MDA levels in plant tissue indicate an increased lipid peroxidation in cell membrane. The high concentration of cellular H_2O_2 and elevated POD activity in our experimental plants suggested that the ROS scavenging system was activated under such stressed conditions. Reduced biomass increment observed in our experiment suggested the plant growth was inhibited. Noticeable declines of *E. nuttallii* populations in Japan (NAGASAKA 2004) and *Elodea canadensis* in Europe (SCULTHORPE 1967) were reported, and scientists have suggested different stress (biotic and abiotic) factors regarding this decline (HAMABATA 1991, KADONO et al. 1997). Our results also supported by BRIX and SORRELL (1996), who reported that wetland plants grown in reducing treatments stopped growing, some of them losing mass.

Conclusions

By subjecting *Elodea nuttallii* to high ammonium concentration in hypoxic/anoxic environments we experienced a number of symptoms, such as suppression of growth, chlorosis of leaves, increased shoot : root ratio, increased lipid peroxidation, decreased proline level, decreased concentrations of mineral cations (such as K, Ca and Mg in the tissues), increased micro elements and decreased photosynthetic pigments etc. Most of these symptoms were reported for ammonium toxicity (BRITTO and KRONZUCKER 2002, CAO et al. 2004) as well as for metal toxicity in a reduced environment (MITTLER 2002, NAGAJYOTI et al. 2010, MONFERRÁN et al. 2012). However, it is difficult to distinguish between high ammonium concentration effect and metal toxicity in a reduced environment. Overall, the combined effect of a low redox state and high ammonium concentration has stronger physiological impact on submerged macrophytes than high ammonium concentration (10 ppm NH_4-N in oxic treatments) acting alone. At the same time the balance of macro-micro nutrients was found more significantly affected by low redox status than by the applied high ammonium concentration in oxic treatment.

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