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Original article

POTENTIAL OF *LEMNOIDEAE* SPECIES FOR PHYTOREMEDIATION OF FRESH WATER WITH ELEVATED MANGANESE CONCENTRATION

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Background. Wastewater treatment using physical, chemical, and biological methods is primary solution for the reduction of water pollution that reaching the critical thresholds. The members of subfamily *Lemnoideae*, commonly called duckweed, are considered the most efficient aquatic plants for wastewater remediation. Although properties of duckweed to survive in water with high concentration of heavy metal ions such as chromium, cobalt, lead, nickel and cuprum are well documented, the growth of duckweed in water with high concentrations of manganese and the efficiency of retention of manganese from water by these species has not been estimated.
Objective. Four duckweed species (*Spirodela polyrhiza*, *Landoltia punctata*, *Lemna aequinoctialis*, and *L. turionifera*) were used for establishment of influence Mn on their vitality and growth and for studying their potential for phytoremediation of fresh water with elevated manganese concentration.
Methods. Duckweed collected in Eastern China was introduced in tissue culture *in vitro* by surface sterilization. The identification of the collected duckweed species was determined by DNA barcoding using primers specific for chloroplast intergenic spacers *atpF-atpH* (ATP) and *psbK-psbL* (PSB). The experiments for establishment of influence Mn on duckweed growth carried out in aseptic condition. To determinate concentration of Mn, the samples of different water type (Hongze Lake, ponds around Hongze Lake, Huaian local municipal sewage plant and industrial sewage plant) were analyzed by the Inductively Coupled Plasma Optical Emission Spectrometry.
Results. The most sensitive duckweed to Mn was *S. polyzhiza*, the first characteristic symptoms of toxicity like brown spots have appeared when concentration of Mn was 40 mg/L, the concentration 200 mg/L Mn resulted in chlorosis and death of fronds. *L. aequinoctialis* and *L. turionifera* had similar effects in SH medium supplemented with 650 mg/L and 975 mg/L Mn, respectively. *L. punctata* was the most tolerant duckweed to Mn plants continued to grow even at concentration 975 mg/L. Response of duckweed on Mn was dependent on availability of nitrogen in nutrient medium. Using four duckweed species for treatment of water containing 4.12 mg/L Mn allowed to reduce concentration until safe level of standard (0.1 mg/L Mn).
Conclusions. All investigated duckweed species (*S. polyrhiza*, *L. punctata*, *L. aequinoctialis*, and *L. turionifera*) were characterized by a high level of resistance to manganese, especially *L. punctata*. Response of duckweed on Mn was dependent on availability of nitrogen in nutrient medium. The tested species of subfamily *Lemnoideae* were high effective for phytoremediation of water with elevated manganese concentration.

Keywords: manganese; phytoremediation; water purification; duckweed; *Spirodela polyrhiza*; *Landoltia punctata*; *Lemna aequinoctialis*; *Lemna turionifera*.

Introduction

Manganese is an abundant element comprising about 0.1% of the earth's crust [1]. Mn is a component of over 100 minerals. Of the heavy metals, it is surpassed in abundance only by iron [2]. Because of the natural release of manganese into the environment by the weathering of manganese-rich rocks and sediments, manganese occurs ubiquitously at low levels in soil, water, air, and food. Dissolved concentrations of manganese in natural waters that are essentially free of anthropogenic sources/influences range from <0.01 mg/L to >10 mg/L [3]. Manganese solubility increases at low pH and under reducing conditions and is most commonly in the 2+ and 4+ oxidation states in aquatic systems.

The presence of chlorides, nitrates and sulphates in high concentrations increase manganese solubility, enhancing aqueous mobility and uptake by plants. [4]. Though manganese can exist in water in any of four oxidation states, Mn(II) is the most common and is usually associated with the carbonate anion CO_3^{2-} to form $MnCO_3$. This compound has a relatively low solubility at 65 mg/L [2]. In the presence of a sufficient amount of oxygen dissolved in water, manganese exists predominantly in an insoluble form (manganese oxide) and is mainly deposited in sediments of water bodies. But, the bottom layers of water usually have lower concentrations of dissolved oxygen. It leads to the development of anaerobic conditions in the deeper layers of the water reservoirs and bottom sediment. In those layers, manganese being converted through bacterial action from insoluble oxide forms to manganese ions (Mn^{2+}) which are soluble and can easily leach out of the sediment into the water. Seasonal and daily heat cycles cause water layers to mix. Therefore, this inversion could be accompanied periodically by a rising concentration of dissolved manganese in drinking water facility intake.

As anthropogenic pollutant manganese present in both inorganic and organic forms. An essential ingredient in steel, inorganic manganese is also used in the production of dry-cell batteries, glass and fireworks, in chemical manufacturing, in the leather and textile industries and as a fertilizer. Organic forms of manganese are used as fungicides, fuel-oil additives, smoke inhibitors, an anti-knock additive in gasoline, and a medical imaging agent [2]. Manganese additives in gasoline are the source of manganese in vehicle emissions. Methylcyclopentadienyl manganese tricarbonyl (MMT) is the main additive containing manganese (approx. 24.4% by weight); the additives LP62 (containing 62% MMT) and LP 46 (containing 46% MMT) are also common [5].

The main target of manganese toxicity is the central nervous system. The neurological effects of inhaled manganese in both humans and animals are reported. Oral doses 1—150 mg/kg of body weight per day resulted in variance in neurotransmitter and enzyme levels in the brain of rats and mice. These changes were accompanied by clinical signs, such as alteration in coordination and activity level [6].

Although manganese is classified under the category of "Data are inadequate for assessment of human carcinogenic potential" by the Environmental Protection Agency (EPA, USA) [7] some new reports include evidences of effects of elevated manganese concentration in drinking water on higher cancer incidence. Based on medical statistic of Cancer Registration System of Huai'an city (China) for 2008 to 2010 years – Huai'an city has a higher cancer incidence. It was demonstrated, that Mn concentration in water sources of Huai'an city area has positive correlation with cancer incidence and mortality: for a 1 μg/L increase in Mn concentration, there was a corresponding increase of 0.45/100000 new cancer cases and 0.35/100000 cancer deaths ($P < 0.05$). [8] The EPA (USA) has established that lifetime exposure to the drinking water with manganese concentration up to 0.3 mg/L is not expected to cause any adverse effects. Besides, maximum manganese concentration for potable water of 0.05 mg/L is established by the U.S. National secondary drinking water standards. In general, a manganese concentration greater than 0.05 mg/L is thought to affect water taste for human. Moreover, manganese concentrations exceeding 0.05 mg/L are sufficient to cause reduced water intake by dairy cattle and, therefore, reduced milk production [9].

A number of physical, chemical, and biological methods are applied in water treatment. Among them biological treatment using aquatic plants especially duckweed has been regarded the most feasible and cost-effective approach for enabling water reuse.

The members of subfamily *Lemnoideae*, commonly called duckweed, are free-floating aquatic plants that are classified into 37 species related to 5 genera based on morphological criteria and employment molecular barcoding techniques [10]. Duckweeds are considered the most efficient aquatic plants for wastewater remediation because they can remove ammonium, nitrates [11], phosphates, heavy metals, arsenic, selenium, boron and organic xenobiotics from different type of wastewater (reviewed in [12]). Besides, wastewater treatment systems based on duckweed are also eco-friendly technique with reduced greenhouse gases emissions [13, 14].

Although properties of duckweed to survive in water with high concentration of heavy metal ions such as chromium, cobalt, lead, nickel and cuprum, and to accumulate substantial amounts of it in the tissue are well documented [15—19], the survival of duckweed in water with high concentrations of manganese and the efficiency of retention of manganese from water by these species has not been estimated.

The aims of the present investigation were: 1) to observe duckweed growth in water with elevated manganese concentrations; 2) to determine manganese concentrations that prevent duckweed growth end cause death for *Lemnoideae* species (*Spirodela polyrhiza*, *Landoltia punctata*, *Lemna turionifera*, *Lemna aequinoctialis*); 3) to evaluate substantial decrease of manganese concentration in water during duckweed growth.

Materials and Methods

Plant material. Four duckweed species, *S. polyrhiza* (N 33''17'40; E 118''49'45), *L. punctata* (N 33''599292, E 119''05674), *L. aequinoctialis* (N 31''14'20; E 121''28'40), and *L. turionifera* (N 33''618817, E 119''001941) used in this study were collected at different locations in Eastern China. Duckweed fronds were surface sterilized in solution containing 0.5% (v/v) sodium hypochlorite solution and 0.1% (v/v) benzalkonium bromide during 5 min, then were washed twice with autoclaved water. The procedure was repeated in 2 days and fronds were put on solid Schenk–Hildebrandt medium (SH) [20]. The composition of nutrient SH medium was 24.73 mM KNO_3, 1.36 mM $CaCl_2$, 1.62 mM $MgSO_4$, 2.6 mM $NH_4H_2PO_4$, 0.42 mkM $CoCl_2$, 0.8 mkM $CuSO_4$, 53.94 mkM Fe-EDTA, 80.86 mkM H_3BO_3, 6.02 mkM KI, 59.17 $MnSO_4$, 0.41 mkM $NaMoO_4$, 3.48 mkM $ZnSO_4$, 5 g/L sucrose, pH was adjusted to 5.5 before autoclaving. The plants were cultivated in incubator at 22 ± 1 °C (for day temperature) and 22 ± 1 °C (for night temperature) with a photon flux density of 50–60 $\mu mol \cdot m^{-2}s^{-1}$ provided by cool white fluorescent bulbs in a 16 h light/8 h dark cycle.

Species identification. The identification of the collected duckweed species was determined by DNA barcoding using primers specific for chloroplast intergenic spacers *atpF-atpH* (ATP) and *psbK-psbL* (PSB) as previously described [10].

Mn treatments. The plant material being grown on solid SH medium supplemented with 5 g/L sucrose was inoculated in 100 mL flasks containing 40 mL autoclaved liquid SH medium or modified SH medium with reduced amount of nitrogen (N_{min}): 2.5 mM KNO_3 instead of 24.73 mM and 2.6 mM KH_2PO_4 as replacement for 2.6 mM $NH_4H_2PO_4$ supplemented with 5 g/L sucrose, pH5.5. The fresh weight of plant material was 50 mg that correspond to 4.6 ± 0.8 mg of the dry weight (DW). To establish influence Mn on vitality and growth of duckweed different amounts of $MnSO_4$ were added in nutrient media (which correspond to the Mn concentration: 3.2, 40, 132, 200, 260, 650, 975 mg/L). After 19 days of growth in aseptic condition in climate chamber the duckweed samples were collected and dried at 60 °C until the weight was constant, then DW was measured. The increase duckweed biomass was calculated as the difference between the measured DW and DW of initial plant material. All experiments were carried out with three replicates.

To investigate phytoremediation potential of different duckweed species for Mn 2g of the plant material was inoculated in plastic basins filled with 200 ml N_{min} medium containing 4.12 mg/L of Mn and cultivated for 19 days in climate chamber. The distilled water was added every 2 days to each container to keep stable water level. All tests were conducted in four repeats for each condition.

Determination of Mn concentration. The samples of different water type were collected from Hongze Lake, ponds around Hongze Lake, Huaian local municipal sewage plant (N 33''629349, E 119''044762) and the industrial sewage plant (N 33''381293, E 118''993329) to determinate concentration of Mn. The samples of 20 ml were digested with equal volume of concentrated HNO_3 in water bath. Then samples were analyzed in triplicates by the Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Perkin Elmer, model: OPTIMA 2000™DV) using winLab32 software. The measurements of Mn concentration were carried out using operating conditions according to [21] with some modifications related to Read Delay (30 seconds), Rinse Delay (30 seconds) and view mode (axial).

Statistical analysis. All experiments were carried out with three biological replicates. The mean values and standard deviation were calculated using MS Office 2019 Excel.

Results

To establish influence Mn on vitality and growth of duckweed species we checked different Mn concentration in nutrient media. Concentration 3.2 mg/L Mn is corresponded to basal salt medium SH and was used as positive control. The highest growth level was observed in N_{min} medium for all tested species. The most sensitive duckweed to Mn was *S. polyzhiza*, the first characteristic symptoms of toxicity like brown spots have appeared when concentration of Mn was 40 mg/L, the concentration 200 mg/L Mn resulted in chlorosis and death of fronds. *L. aequinoctialis* and *L. turionifera* had similar effects in SH medium supplemented with 650 mg/L and 975 mg/L Mn, respectively. *L. punctata* was the most tolerant duckweed to Mn, plants continued to grow even at concentration 975 mg/L (Table 1, the Figure). Obtained data demonstrated that duckweed species are sufficiently resistant to Mn.

To assess the growth of duckweed after 19 days of cultivation on different media, the fronds were collected, dried, and weighed, and the increase biomass was calculated as the difference between the measured DW and DW of initial plant material (Table 1).

Table 1: Increase of duckweed biomass DW after 19 days growth on different media (mg)

Mn, mg/L	*S. polyzhiza*		*L. aequinoctialis*		*L. turionifera*		*L. punctata*	
	N_{min}	SH	N_{min}	SH	N_{min}	SH	N_{min}	SH
3.2	121.3 ± 5.8	95.2 ± 5.3	111.4 ± 12.1	79.6 ± 9.6	126.4 ± 5.6	104 ± 9.6	113.0 ± 10.2	110.0 ± 5.6
40	110.0 ± 6.4*	102.6 ± 11.2	122.3 ± 2.3	73.0 ± 7.2	105.3 ± 12.1	65.2 ± 7.2	98.6 ± 1.4	113.6 ± 6.2
132	116.2 ± 6.2*	89.8 ± 5.8	110.6 ± 11.0	70.6 ± 8.6	107.0 ± 7.6*	73.5 ± 5.5	93.4 ± 5.4	97.8 ± 8.1
200	29.2 ± 7.9**	31.3 ± 7.0**	116.4 ± 10.4	75.2 ± 12.2	122.2 ± 10.6*	89.0 ± 7.6	85.6 ± 5.8	73.4 ± 7.4
260	17.3 ± 7.4**	20.6 ± 9.2**	72.4 ± 12.3*	68.2 ± 7.2	97.8 ± 7.8*	83.4 ± 12.6	83.3 ± 5.3	70.6 ± 4.2
650	n.a.	n.a.	23.8 ± 4.4**	11.6 ± 4.8**	28.4 ± 5.4*	26.4 ± 6.8	41.3 ± 7.1	36.4 ± 10.2
975	n.a.	n.a.	19.8 ± 5.4**	9.8 ± 5.6**	26.2 ± 8.2**	13.2 ± 4.8**	36.8 ± 14.2	35.8 ± 9.8

Notes. The increase duckweed biomass was calculated as the difference between the measured DW and DW of initial plant material. Data are means ±SD of three replicates. * – brown spots on single fronds; ** – more than 90% dead fronds; n.a. – not applicable.

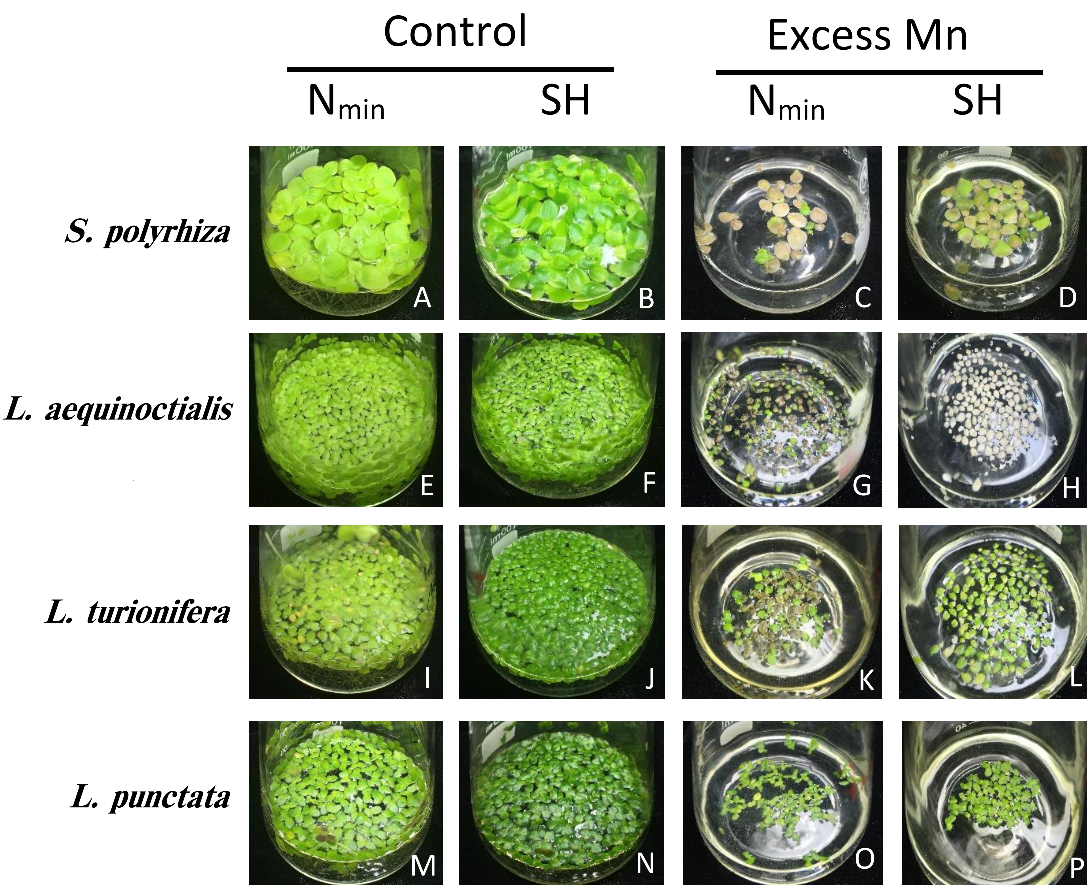

Figure: Influence of different media on growth of duckweed: *S. polyrhiza (A-D)*, *L. aequinoctialis (E-H)*, *L. turionifera (I-L)*, *L. punctate (M-P)*. A, E, I, M – control medium N_{min} with 3.2 mg/L Mn, B, F, J, N – control medium SH with 3.2 mg/L Mn, C – medium N_{min} with 40 mg/L Mn, D – medium SH with 40 mg/L Mn, G, K – medium N_{min} with 650 mg/L Mn, H, L – medium SH with 650 mg/L Mn, O – medium N_{min} with 975 mg/L Mn, P – medium SH with 975 mg/L Mn

Another aim of our work was to check the Mn concentration in water of various origin (see Materials and Methods). Analysis of samples of different kinds of water by using ICP-OES revealed anthropogenic Mn pollution of ponds around Hongze Lake. Mn content in one of the ponds was almost in 20 times higher than the standard for drinking water (1.96 in comparison with 0.1 mg/L, China Standard GB3838-2002) (Table 2).

Determination of Mn concentration in media after 19-days growth of duckweed demonstrated that plants took up about 98% of the Mn in condition of our testing. The initial concentration of Mn dropped down from 4.12 mg/L to 0.097 mg/L for

L. aequinoctialis, to 0.0735 mg/L for *S. polyrhiza*, to 0.078 mg/L for *L. Punctata*, and to 0.0995 mg/L for *L. turionifera*. All duckweed species were able to decrease the Mn concentration to standart of drinking water (0.1 mg/L). The obtained results show that all tested duckweed species have high potential for Mn phytoremediation.

Table 2: Concentration of Mn in different kinds of water

Water samples	Concentration of Mn, mg/L
Standard	0.100
Tap water	0.040
Hongze Lake	0.063
Pond around Hongze Lake	1.958
Municipal wastewater OUT	0.060
Industrial wastewater OUT	0.325

Discussion

Manganese (Mn) is cofactor for proteins that have crucial role in photosynthesis, the metabolism of fatty acids and carbohydrates as well as in protection against oxygen free radicals. Therefore, deficiency of this essential nutrient is causes interveinal chlorosis and reduced plant biomass [22]. On the other hand, excess Mn concentrations can be toxic for plants resulting in brown spots on mature leaves [23], chlorosis, and necrosis and deformation of young leaves [24]. The threshold of Mn toxicity varies according to plant species or cultivars [24]. Among tested duckweed species *L. punctata* was the most resistant to high concentration of Mn, *S. polyzhiza* was the most sensitive. In spite of difference between species all of them can be attributed to Mn-tolerant plants.

The general trend followed from our experiments was that vitality of duckweed in presence of excess Mn concentrations was higher in media with low concentration of nitrogen (N_{min}). Different response of duckweed on the same Mn concentration can indicate indirectly on the cross-talk between uptake of Mn and nitrogen.

Symptoms of Mn toxicity were also dependent on composition of nutrient medium. Plants growing on N_{min} medium with low content of nitrogen had brown spots which magnified with increasing Mn concentration leading to general necrosis of fronds. Whereas plants growing on SH medium have demonstrated chlorosis which started from apex and spread to whole frond. Obtained data and discovered patterns lay the foundation for further investigation of mechanism of Mn phytotoxicity and tolerance at molecular level.

Analysis of the concentration of Mn in various types of water revealed anthropogenic pollution of ponds. One of tested ponds had concentration of Mn almost 20 times higher than the standard for drinking water. Using duckweed for treatment of water containing even higher concentration of Mn allowed to reduce it below of the safe level of standard that allows to characterize duckweed as high effective plant for water remediation.

Conclusions

Analysis of different kind of water by ICP-OES showed that manganese pollution of ponds around Hongze Lake are reached the critical thresholds and water treatment measures are required. In condition of our experiments the tested species of subfamily *Lemnoideae* were high effective for phytoremediation of water with elevated manganese concentration and were able to decrease the Mn concentration to standart of drinking water: the initial concentration of Mn was reduced from 4.12 mg/L to 0.0734–0.097 mg/L.

All investigated duckweed species (*S. polyrhiza*, *La. punctata*, *L. Aequinoctialis*, and *L. turionifera*) were sufficiently resistant to manganese, especially *L. punctata* which vegetative grown even at concentration of Mn 975 mg/L. Duckweed response on Mn was dependent on concentration of nitrogen in nutrient medium. The same Mn concentration can lead to various effects namely either necrosis or chlorosis according to concentration of nitrogen in medium. These observations point indirectly on the crosstalk between uptake of Mn and nitrogen. The survival of duckweed in presence of excess Mn concentrations was also dependent to concentration of nitrogen. It was demonstrated that duckweed fronds better survived in presence of excess Mn in media with low concentration of nitrogen. Represented data show that duckweed is a convenient model for further investigation of both Mn metabolism at the molecular level and the mechanism occurrence of phytotoxicity.

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ПОТЕНЦІАЛ ПРЕДСТАВНИКІВ ПІДРОДИНИ *LEMNOIDEAE* ДЛЯ ФІТОРЕМЕДІАЦІЇ ПРІСНОЇ ВОДИ ІЗ ПІДВИЩЕНОЮ КОНЦЕНТРАЦІЄЮ МАРГАНЦЮ

Проблематика. Очищення стічних вод із використанням фізичних, хімічних і біологічних методів є основним рішенням для зниження забруднення води, яке часом досягає критичних порогів. Члени підродини *Lemnoideae*, яких зазвичай називають ряскою, вважаються найбільш ефективними водними рослинами для очищення стічних вод. Хоча властивості ряски виживати у воді з високою концентрацією іонів важких металів, таких як хром, кобальт, свинець, нікель і мідь, добре задокументовані, однак про ріст ряски у воді з високими концентраціями марганцю та про ефективність видалення марганцю з води цими видами повідомлень не було.
Мета. Чотири види ряски (*Spirodela polyrhiza*, *Landoltia punctata*, *Lemna aequinoctialis* і *L. turionifera*) були використані для встановлення впливу Mn на їх життєздатність і ріст, а також для вивчення їх здатності до фіторемедіації прісної води з підвищеною концентрацією марганцю.
Методика реалізації. Ряску, зібрану у водоймах Східного Китаю, вводили в культуру тканин *in vitro* поверхневою стерилізацією. Ідентифікацію зібраних видів ряски проводили за допомогою штрихкодування ДНК із використанням праймерів, специфічних для хлоропласних міжгенних спейсерів *atpF-atpH* (ATP) і *psbK-psbL* (PSB). Експерименти зі встановлення впливу Mn на ріст ряски проводили в асептичних умовах. Для визначення концентрації Mn зразки різних типів води (озеро Гонзе, ставки біля озера Гонзе, місцева комунальна каналізаційна станція Хуайяна та станція промислових стічних вод) було проаналізовано за допомогою методу атомно-емісійної спектрометрії з індуктивно-зв'язаною плазмою.
Результати. Найчутливішою ряскою до Mn була *S. polyzhiza*. Перші характерні симптоми токсичності, такі як коричневі плями, з'явились, коли концентрація Mn становила 40 мг/л, концентрація 200 мг/л Mn призводила до хлорозу та загибелі листеців. *L. aequinoctialis* і *L. turionifera* мали подібний ефект при вирощуванні в середовищі SH, доповненому 650 і 975 мг/л Mn відповідно. *L. punctata* була найбільш стійкою серед рясок до Mn, вона не припиняла ріст навіть за концентрації 975 мг/л. Відповідь ряски на Mn залежала від доступності азоту в живильному середовищі. Аналіз концентрації Mn у різних видах води показав антропогенне забруднення ставків навколо озера Гонзе, в одному з них вміст Mn майже в 20 разів перевищує стандарт для питної води (1,96 замість 0,1 мг/л). Використання 4-х видів ряски для очищення води, що містила 4,12 мг/л Mn, дало можливість зменшити концентрацію до безпечного рівня стандарту (0,1 мг/л Mn).
Висновки. Всі досліджуванні види ряски (*S. polyrhiza*, *L. punctata*, *L. aequinoctialis* і *L. turionifera*) характеризувалися високим рівнем стійкості до марганцю, особливо *L. punctata*. Реакція ряски на Mn залежала від концентрації азоту в живильному середовищі. Випробовувані види підсімейств *Lemnoideae* були високоефективними для фіторемедіації води з підвищеною концентрацією марганцю.

Ключові слова: марганець; фіторемедіація; очищення води; ряска; *Spirodela polyrhiza*; *Landoltia punctata*; *Lemna aequinoctialis*; *Lemna turionifera*.

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ПОТЕНЦИАЛ ПРЕДСТАВИТЕЛЕЙ ПОДСЕМЕЙСТВА *LEMNOIDEAE* ДЛЯ ФИТОРЕМЕДИАЦИИ ПРЕСНОЙ ВОДЫ С ПОВЫШЕННОЙ КОНЦЕНТРАЦИЕЙ МАРГАНЦА

Проблематика. Очистка сточных вод с использованием физических, химических и биологических методов является основным решением для снижения загрязнения воды, которое порой достигает критических порогов. Члены подсемейства *Lemnoideae*, которых обычно называют ряской, считаются наиболее эффективными водными растениями для очистки сточных вод. Хотя свойства ряски выживать в воде с высокой концентрацией ионов тяжелых металлов, таких как хром, кобальт, свинец, никель и медь, хорошо задокументированы, о росте ряски в воде с высокими концентрациями марганца и об эффективности удаления марганца из воды этими видами сообщений не было.
Цель. Четыре вида ряски (*Spirodela polyrhiza, Landoltia punctata, Lemna aequinoctialis и L. turionifera*) были использованы для установления влияния Mn на их жизнеспособность и рост, а также для изучения их способности к фиторемедиации пресной воды с повышенной концентрацией марганца.
Методика реализации. Ряску, собранную в водоемах Восточного Китая, вводили в культуру тканей *in vitro* путем поверхностной стерилизации. Идентификацию собранных видов ряски проводили с помощью штрихкодирования ДНК с использованием праймеров, специфичных для хлоропластных межгенных спейсеров *atpF-atpH* (ATP) и *psbK-psbL* (PSB). Эксперименты по установлению влияния Mn на рост ряски проводили в асептических условиях. Для определения концентрации Mn образцы различных типов воды (озеро Гонзо, пруды у озера Гонзо, местная коммунальная канализационная станция Хуайяна и станция промышленных сточных вод) были проанализированы с помощью метода атомно-эмиссионной спектрометрии с индуктивно-связанной плазмой.
Результаты. Самой чувствительной ряской к Mn была *S. polyzhiza*. Первые характерные симптомы токсичности, такие как коричневые пятна, появились, когда концентрация Mn составляла 40 мг/л, концентрация 200 мг/л Mn приводила к хлорозу и гибели листецов. У *L. aequinoctialis* и *L. turionifera* подобный эффект наблюдался при выращивании в среде SH с добавлением 650 и 975 мг/л Mn соответственно. *L. punctata* была наиболее устойчивой среди рясок к Mn, она не прекращала рост даже при концентрации 975 мг/л. Ответ ряски на Mn зависел от доступности азота в питательной среде. Анализ концентрации Mn в разных видах воды показал антропогенное загрязнение прудов вокруг озера Гонзо, в одном из них содержание Mn почти в 20 раз превышало стандарт для питьевой воды (1,96 вместо 0,1 мг/л). Использование 4 видов ряски для очистки воды, содержащей 4,12 мг/л Mn, позволило уменьшить концентрацию до безопасного уровня стандарта (0,1 мг/л Mn).
Выводы. Все исследованные виды ряски (*S. polyrhiza*, *L. punctata*, *L. aequinoctialis* и *L. turionifera*) характеризовались высоким уровнем устойчивости к марганцу, особенно *L. punctata*. Реакция ряски на Mn зависела от концентрации азота в питательной среде. Испытуемые виды подсемейства *Lemnoideae* были высокоэффективны для фиторемедиации воды с повышенной концентрацией марганца.

Ключевые слова: марганец; фиторемедиация; очистка воды; ряска; *Spirodela polyrhiza*; *Landoltia punctata*; *Lemna aequinoctialis*; *Lemna turionifera*.