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The inhibition and adaptability of four wetland plant species to high concentration of ammonia wastewater and nitrogen removal efficiency in constructed wetlands

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HIGHLIGHTS

- Four wetland plant species growth study with high TAN concentration was performed.
- Physiological and enzymatic responses were studied in CWs microcosm.
- *Canna indica* and *Typha orientalis* had stronger adaptability in TAN-rich solution.
- Low TAN concentration as nitrogen source stimulated plant growth.
- TAN caused cellular level disorder with a threshold for self-bioremediation.

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ABSTRACT

Four plant species, *Typha orientalis*, *Scirpus validus*, *Canna indica* and *Iris tectorum* were selected to assess their physiological response and effects on nitrogen and COD removal to high total ammoniacal nitrogen (TAN) in constructed wetlands. Results showed that high TAN caused decreased relative growth rate, net photosynthetic rate, and leaf transpiration. *C. indica* and *T. orientalis* showed higher TAN adaptability than *S. validus* and *I. tectorum*. Below TAN of 200 mg L^{-1}, growth of *C. indica* and *T. orientalis* was less affected or even stimulated at TAN range 100–200 mg L^{-1}. However, *S. validus* and *I. tectorum* was obviously suppressed when TAN was above 100 mg L^{-1}. High TAN generated obvious oxidative stress showing increased proline and malondialdehyde contents, and superoxide dismutase was inhibited. It indicated that the threshold for plant self-bioremediation against high TAN was 200 mg L^{-1}. What's more, planted CWs showed higher nitrogen and COD removal. Removal rate of *C. indica* and *T. orientalis* was higher than *S. validus* and *I. tectorum*.

1. Introduction

In wastewater from livestock holding and slaughtering plants, the ammonia concentration usually ranges from 400 to 500 mg L^{-1} (Clarke and Baldwin, 2002). The primary negative impacts of ammonia on aquatic environment include degradation of water bodies through depressed dissolved oxygen (DO) level, thus causing aquatic biota toxicity (Horne, 2001). As a sustainable and energy-efficient method, constructed wetlands (CWs) are a viable technology for nitrogen pollution treatment. CWs have the advantages of easy maintenance, on-site treating and good self-purification capacity, and are widely practiced for treating wastewaters from concentrated agro-industrial animal production sectors in many small communities and rural area, especially where integrated wastewater treatment plants are unavailable (Kadlec and Knight, 1996; Vymazal, 2011).

In CWs, nearly 60–70% of total nitrogen is removed by means of de-nitrification, while 20–30% can be removed by plant uptake (Spieles and Mitsch, 2000). Although the removal rate of nitrogen by plant uptake seems obviously lower than that of microorganisms, the key roles of plants in CWs include the reduction of wind speed, prevention of re-suspension, provision of enormous surface areas for accreted microorganisms, transformation of oxygen to rhizosphere, and secretion of organic carbon to surroundings (Marchand et al., 2010).

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Ammonium and nitrate are the major inorganic nitrogen sources for wetland plants. NH_4-N can be easily assimilated due to the low energy cost (Miller and Cramer, 2005). However, it is known that total ammoniacal nitrogen (TAN) above a concentration, which is dependent on wetland plant species, is detrimental to macrophytes, thus indirectly reducing the removal efficiency of these pollutants. Free ammonia is the main inhibitory form of TAN (Boussiba and Gibson, 1991). Its inhibitory effect is caused by free penetration through cell membranes and poisoning the plant photosynthetic system (Abeliovich and Azov, 1976). Excessive TAN disturbs nutrient uptake and hormone balance, reduces the content of soluble carbohydrates, increases the free amino acid level, and negatively affects metabolism and photosynthesis (Britto and Kronzucker, 2002). The symptoms of ammonia toxicity include inhibition of growth, chlorosis of leaves, reduction of concentrations of mineral cations, and enhancement of anions in tissues. High concentration of TAN may induce osmotic stress and reactive oxygen species (ROS) accumulation, including superoxide radical and hydrogen peroxide, which can cause productivity decrease, injury and even death of the plant cells through damaging lipids and protein (Wang et al., 2010).

Plant species differ widely in their toxicity tolerance against high TAN level. The ability of wetland plants to cope with high TAN wastewater is of special interest considering plant usage in CWs. For instance, *Canna indica* was reported to be more efficient to purify domestic wastewater than *Typha latifolia* due to its better tolerance to ammonia (Yang et al., 2007). Species such as duckweeds, *Lemna* and *Azolla*, water hyacinth, *Eichhornia crassipes*, have been successfully tested for their tolerance to high concentration of TAN. *Typha orientalis*, *Scirpus validus*, *C. indica* and *Iris tectorum* also have the application potential in CWs as they have high growth rate in nutrient-rich and stagnant waters. Although CWs plants have been identified according to their growth characteristics (Clarke and Baldwin, 2002; Liu et al., 2012), the detailed plant growth and physiological responses to high TAN exposure or their effects in treating high TAN wastewater is still unknown. Moreover, the variation of plant responses to high TAN concentration wastewater with time, and their adaptability to such concentration level have not been deeply studied. A better understanding of the time-dependent physiological responses of wetland plant to high TAN concentrations is required for optimizing the selection, management and application of these plants in purifying high TAN wastewater.

Hence, in this study, growth and physiological responses of four commonly applied wetland plants *T. orientalis*, *S. validus*, *C. indica*, and *I. tectorum* to the high TAN exposure were reported with the aim of indicating suitable CWs plant species for efficient treatment of wastewaters containing high TAN. Firstly, inherent rules of growth and physiological indexes by factor analysis were determined. Detailed time-dependent measurement with TAN dose-response of relative growth rate (RGR), photosynthetic rate (Pn) and transpiration rate (Tr), proline (Pro), malondialdehyde (MDA) contents, and superoxide dismutase (SOD) activity were analyzed. Then, the removal efficiency of pollutants in effluents by different plant species were compared. Symptoms, effects, and possible mechanisms of ammonia toxicity of plants in the treatment of high TAN wastewater were discussed.

2. Methods

2.1. Experimental set-up

The study was carried out in Donghua University, Shanghai. Twenty polyethylene plastic containers (0.5 m × 0.35 m × 0.3 m), used as microcosm horizontal subsurface flow constructed wetlands (HSCWs), were filled with approximately 0.25 m high uniform quartz sand (φ = 3–6 mm). The containers for each plant were divided into 4 groups including controls and each group contained 4 replicates (n = 4). Disease-free plant seedlings of *T. orientalis*, *S. validus*, *C. indica*, and *I. tectorum* at similar growing potential were purchased from a local flower market. Initial stem lengths of the four macrophytes were measured to be 40 ± 5 cm, 20 ± 3 cm, 30 ± 6 cm, and 23 ± 3 cm, respectively. Before experiments, plants were propagated for a three-week acclimation in culture solution in buckets. The ingredients of culture solution contained 1.0% Hoagland's trace elements and marco-nutrient elements (Hoagland and Arnon, 1938), including 945 mg L^{-1} Ca $(NO_3)_2{\cdot}4H_2O$, 506 mg L^{-1} KNO_3, 136 mg L^{-1} KH_2PO_4, and 493 mg L^{-1} $MgSO_4$. After acclimation, the plants were rinsed for 3 times with de-ionized water and then transplanted to HSCWs. The plants were employed in the container at a density of 6 rhizomes per unit.

The plants were exposed to synthetic high TAN wastewater for 35 days. TAN concentrations were 0 (control), 100, 200, 300, and 400 mg L^{-1} (N) ammonium chloride (NH_4Cl). Other ingredients were composed of 27.5 mg L^{-1} $MgSO_4{\cdot}7H_2O$, 2.5 mg L^{-1} $CaCl_2$ and 20 mg L^{-1} KNO_3 in de-ionized water and stored in a lighttight tank. The pH of the NH_4Cl stock solution was adjusted with HCl/NaOH to the pH = 7.5 ± 0.3 in the control/cultivation medium, in order to avoid pH variation in exposures. The exposure solution was exchanged every 2 days in order to maintain TAN level and pH.

The exposure experiment began on April 1, 2014 and lasted for 35 days. The air temperature ranged from 13 to 21 °C. The plant leaf samples were harvested at a 5-day interval. Four sample leaves were randomly ground from each reactor. Each sample was fully mixed before analysis. The plant leaves were rinsed separately with distilled water and immediately frozen in liquid nitrogen for storage at −80 °C.

2.2. Determination of plant growth

Growth-related indices were used to evaluate the tolerance of plant species to high TAN. A higher net photosynthetic rate (Np) was important to the amount of N and P uptake and determined the faster biomass production, whereas a higher leaf transpiration rate (Tr) also facilitated the transport of N from root to shoot (Alam, 1999). The Np and Tr were measured by a portable infrared gas analyzer (LI-6400XT Portable Photosynthesis System, LI-COR, USA). The flow rate of CO_2 in the chamber was adjusted to 400 μmol s^{-1}. The light intensity was fluxed by red-blue LED light source in the chamber at 1000 μmol m^{-2} s^{-1}. The leave area was measured to be 6 cm^2. Biomass of plants in each container were weighed and recorded as dry weight at the end of the inhibition on day 35. The plants were rinsed with distilled water and blotted with tissue paper. Relative growth rate (gg^{-1} d^{-1}) in treatment was calculated as: $RGR = (\ln W_2 - \ln W_1)/t$, where W_1 and W_2 were the initial and final dry weight (g) and t was the incubation time (days).

2.3. Contents of free proline and MDA and SOD activity

Leaf biochemical response indices were used to present the inhibition from high TAN concentration. Pro content of leaves was extracted and tested with the modified method of Bates et al. (1973). Firstly, 0.5 g fresh material was prepared with 5 mL of aqueous sulfosalicylic acid (3% w/v) in a boiling tube and homogenized with a pulp refiner. Then, the solution was put in a water bath at 100 °C for 10 min and then cooled down with tap water. The mixture was centrifuged at 2500 rpm for 10 min. The supernatant (2 mL) was reacted with 2 mL of glacial acetic acid and 3 mL of ninhydrin reagent and then incubated at 95 °C for