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Copper uptake and translocation in a submerged aquatic plant *Hydrilla verticillata* (L.f.) Royle

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ABSTRACT

A comprehensive understanding of the uptake, tolerance and transport of heavy metals in the wetland system through aquatic plants will be essential for the development of phytoremediation technologies. Copper accumulation and translocation of a submersed macrophyte *Hydrilla verticillata* (L.f.) Royle were investigated. Plant shoots showed a significant accumulation of Cu with a maximum of 30 830 mg Cu kg^{-1} dry weight after exposed to 4000 μg L^{-1} Cu for 4 d. Both roots and shoots can directly take up Cu from solution and Cu mainly accumulated in cell wall fractions. Moreover, *H. verticillata* predominantly accumulated Cu through shoots from the aqueous solutions because of the higher weights and bioaccumulation factors of shoots than those of roots. Acropetal translocation of Cu in the plant is higher than the basipetal translocation, which implies that upward translocation of Cu is mainly via the xylem and downward translocation is mainly through the phloem. These findings contribute to the application of submerged aquatic plants to copper removal from moderately contaminated waters.

1. Introduction

The pollution of aquatic ecosystems by heavy metals has been attracting considerable public attention over the past few decades (Nriagu and Pacyna, 1988; Rai, 2009). Copper is a ubiquitous metal present in the environment through natural and anthropogenic pathways (Aksu and Donmez, 2000; Savvaidis et al., 2003). The average concentration of Cu in polluted waters ranges from 30 to 60 μg L^{-1} (Brown and Rattigan, 1979). However, the concentration of Cu ions in industrial wastewaters can be up to 1000 mg L^{-1} (Figueira et al., 2000).

Although it is an essential microelement for plant growth, elevated concentrations of Cu can cause toxicity to plants (Mal et al., 2002; Yruela, 2009). Traditional technologies for cleaning contaminated waters have been proven to be efficient, but not cost-effective. Phytoremediation, a plant-based green technology, has appeared as a promising alternative and cost-effective method for metal removal from moderately contaminated waters (Weis and Weis, 2004; Rai, 2009). Submersed macrophytes, which play important structural and functional roles in aquatic ecosystems (Arts et al., 2008), have shown tremendous potential to accumulate metals (Cardwell et al., 2002; Peng et al., 2008), and can be used to remove heavy metals from stormwaters (Fritioff et al., 2005) and secondarily treated wastewaters (Keskinkan, 2005). When metals are absorbed by plants, they can be trapped by the negative charges of the cell walls or be taken up into the cell cytoplasm and compartmented in some nonfunctional organs like vacuoles, or they can be extracellularly excreted (Hall, 2002). However, the mechanisms of tolerance to heavy metals in submerged macrophytes are still not clear.

Since submerged plants are exposed to both overlying water and sediment, they are able to absorb nutrients and chemicals from both environments (Hinman and Klaine, 1992). The general view is that roots are thought to be important for element uptake in freshwater macrophytes (Jackson, 1998). Fritioff and Greger (2006) found that Cu can be taken up by the leaves, stems, and roots of *Potamogeton natans*, with the highest accumulation found in the roots. After an investigation of 15 species of aquatic macrophytes living in contaminated urban streams of southeast Queensland, Cardwell et al. (2002) found that roots accumulated higher metal concentrations than other tissues. However, since roots of submerged macrophytes are degenerated and greatly reduced in size (Basiouny et al., 1977), their potential for metal uptake may be limited. In addition, shoots have the ability to take up metals directly from water since they are completely inundated and have a very thin cuticle (Jackson, 1998). Thus it is possible that shoot uptake is the primary pathway when high Cu levels are present in the water.

Since submersed aquatic angiosperms have a reduced vascular system and lack a transpiration stream (Hinman and Klaine,

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1992), it has been questioned whether translocation of metals occurs. No translocation of Cu from root to other parts was found in *P. natans* (Fritioff and Greger, 2006), but on the study of *Potamogeton pectinatus*, Cu can be acropetally transported with shoot to root concentration ratio $<10^{-4}$ (Wolterbeek and van der Meer, 2002), and this is congruent with previous results concerning Cu translocation in *P. pectinatus* and *Potamogeton crispus* (Peter et al., 1979). It can be seen that translocation ability of metals is different between plant species. Metals may be translocated via the apoplast, in the phloem, and acropetally in the xylem of plants (Peter et al., 1979). In *Elodea canadensis*, it has been suggested that Cd translocation is apoplastic (Fritioff and Greger, 2007). However, the process of Cu translocation in submerged macrophytes is less well understood.

Hydrilla verticillata is a common aquatic angiosperm, which has worldwide distribution and grows at a rapid rate, and it has been established as a potential accumulator of heavy metals like Pb, Hg, Cu, Cd, Cr, Ni and As (Gupta and Chandra, 1994; Gupta et al., 1996; Sinha and Pandey, 2003; Srivastava et al., 2007). However, little information is available on mechanisms of Cu tolerance and translocation in this plant. Thus, the objectives of our study were: (i) to investigate Cu uptake and tolerance by *H. verticillata*; (ii) to determine Cu translocations in both upward and downward directions, as well as to study its subcellular localization in order to manifest the distribution and translocation paths among different tissues, and (iii) to explore the Cu efflux potential from shoots to the water environment.

2. Materials and methods

2.1. Plant growth

Ten centimeter long vegetative top shoots of *H. verticillata* (L.f.) Royle, were obtained from Lake Taihu, Wuxi, China. The shoots were planted in the greenhouse pond (filled with tap water, the substrate of 50% soil and 50% sand) until new vegetative shoots and roots had developed. Before Cu exposure, plants (about 3 cm tip portion for the uptake and toxicity experiments; and whole plants with roots and shoots for the translocation experiments) were acclimatized for 7 d in laboratory conditions: 115 μmol m^{-2} s^{-1} light with a 14 h photoperiod at 25 ± 2 °C. The composition (mg L^{-1}) of the synthetic fresh water was as follows: 22.7 $MgSO_4{\cdot}7H_2O$, 30.7 $MgCl_2{\cdot}2H_2O$, 20.4 $CaCl_2{\cdot}2H_2O$, 45.7 NaCl, 26.0 $NaHCO_3$, 3.61 KCl, 1.41 $FeCl_3{\cdot}6H_2O$, 0.97 $Al_2(SO_4)_3{\cdot}18H_2O$, 0.19 $MnCl_2{\cdot}4H_2O$, (μg L^{-1}) 3.86 $ZnSO_4{\cdot}7H_2O$, 2.17 $CuCl_2{\cdot}2H_2O$ (pH adjusted to 7.0 with NaOH or HCl solutions) (Stauber and Florence, 1989). Free cupric ion (Cu^{2+}) is the predominant species of Cu (>50%) in fresh water solutions (Markich et al., 2006).

2.2. Copper toxicity

The toxicity of Cu to *H. verticillata* was tested using the protocol described by Markich et al. (2006). Three replicates of 1 g *H. verticillata* (three plants having fresh apical shoots with similar size of 20–30 mm) were cultured in a jar (10 cm in diameter and 10 cm in depth) containing 500 mL synthetic freshwater and different concentrations of Cu (up to 4000 μg L^{-1}), supplied as $CuCl_2{\cdot}2H_2O$. Each container was divided into four equal sections with polypropylene mesh (8 × 10 mm open grids), and three plants were placed in each container with an air stone placed into the remaining section of each container to ensure constant dissolved oxygen levels. The solutions were renewed every 2 d. After 4 d the plants were harvested, washed carefully with de-ionized water and blotted dry. Each plant was weighted (FW) and stem length was measured. The samples were oven-dried and weighed to determine the dry weight (DW). The total concentrations of Cu in shoots of plants were determined (see Section 2.6).

2.3. Copper translocation

Intact plants were grown with roots and shoots in separate containers (Fig. 1) described by Fritioff and Greger (2007) with a slight modification during the experiment. The containers (volume 1.8 L with height 7 cm × width 9 cm × length 28 cm) were filled with 1.05 L synthetic freshwater medium (with 250 mL in the root chamber and 800 mL in the shoot chamber) and covered with a membrane with small holes to minimize evaporation. In addition, a layer of water-moistened gauze was placed on the air-exposed portion of the shoot in the small gap between containers to avoid the desiccation of air-exposed plant parts.

Both acropetal and basipetal translocations of Cu at different exposure times were examined in *H. verticillata*. Each root or shoot was exposed to 128 μg L^{-1} Cu solution in separate containers, except in the case of the controls. There were four replicates in each treatment and each replicate consisted of two plants. Plants were sampled periodically up to 96 h Cu exposure to investigate Cu transportation in plants and subcellular distributions of Cu in different tissues. Each plant was cut between containers, washed carefully with de-ionized water, and then divided into leaves, stems, roots, and air-exposed parts were removed. The samples were blotted dry and fresh weights (FW) were recorded, and frozen in liquid N_2 until use.

2.4. Copper efflux from H. verticillata to solution

Shoots of plants (two plants per vessel) were exposed to 50 mL solutions containing 128 μg L^{-1} Cu. After 24 h, plants were collected and rinsed in the solution (5 mM EDTA and 5 mM 2-(*N*-morpholino)ethanesulfonic acid pH 6.0) for 5 min to remove Cu adsorbed on the cell walls (Hassler et al., 2004). Shoots were then transferred to a new vial containing double de-ionized water without Cu and Cu concentrations in the efflux solution were analyzed periodically up to 24 h exposure.

2.5. Fractioning

Frozen materials were homogenized in pre-chilled extraction buffer (comprising 50 mM Hepes, 500 mM sucrose, 1.0 mM dithiothreitol, 5.0 mM ascorbic acid, and 1.0% (w/v) polyvinyl polypyrrolidone, adjusted to pH 7.5 with NaOH) with a chilled mortar and pestle. Cells were separated into three fractions: cell wall, soluble fraction and organelle containing fraction using differential centrifugation technique as suggested by Lozano-Rodrı′guez et al. (1997) with some modifications.

The homogenate was sieved through a nylon cloth (100 μm mesh size) and washed with extraction buffer, this residue, together with the pellet retained after centrifugation of the filtrate

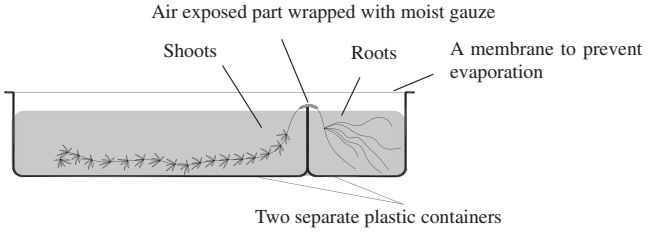

Fig. 1. Experimental set-up in a two-container system to investigate uptake and subcellular distributions in different tissues of an intact *H. verticillata* plant and translocation of copper from one tissue to another.