Environmental Pollution 187 (2014) 22–30

Contents lists available at ScienceDirect

Environmental Pollution

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

Evidence of sulfur-bound reduced copper in bamboo exposed to high silicon and copper concentrations

Blanche Collin [a,b,*,1], Emmanuel Doelsch [c], Catherine Keller [a], Patrick Cazevieille [c], Marie Tella [c], Perrine Chaurand [a], Frédéric Panfili [b], Jean-Louis Hazemann [d], Jean-Dominique Meunier [a]

[a] CEREGE, CNRS, Aix-Marseille Univ., Europôle méditerranéen de l'Arbois, BP 80, 13545 Aix en Provence, France
[b] Département de recherche développement de la société PHYTOREM S.A., Chemin de l'Autodrome, 13140 Miramas, France
[c] CIRAD, UPR Recyclage et risque, Avenue Agropolis, F-34398 Montpellier, France
[d] Institut Néel, CNRS and Université Joseph Fourier, BP 166, F-38042 Grenoble Cedex 9, France

ARTICLE INFO

Article history:
Received 9 September 2013
Received in revised form
13 December 2013
Accepted 23 December 2013

Keywords:
Metal
Toxicity
Phytoremediation
Poaceae
X-ray absorption spectroscopy

ABSTRACT

We examined copper (Cu) absorption, distribution and toxicity and the role of a silicon (Si) supplementation in the bamboo *Phyllostachys fastuosa*. Bamboos were maintained in hydroponics for 4 months and submitted to two different Cu (1.5 and 100 μm Cu^{2+}) and Si (0 and 1.1 mM) concentrations. Cu and Si partitioning and Cu speciation were investigated by chemical analysis, microscopic and spectroscopic techniques. Copper was present as Cu(I) and Cu(II) depending on plant parts. Bamboo mainly coped with high Cu exposure by: (i) high Cu sequestration in the root (ii) Cu(II) binding to amino and carboxyl ligands in roots, and (iii) Cu(I) complexation with both organic and inorganic sulfur ligands in stems and leaves. Silicon supplementation decreased the visible damage induced by high Cu exposure and modified Cu speciation in the leaves where a higher proportion of Cu was present as inorganic Cu(I)S compounds, which may be less toxic.

1. Introduction

Phytoremediation technologies are currently used for wastewater treatment (McCutcheon and Schnoor, 2003; Schroder et al., 2007; Vymazal, 2011). Bamboos are known for their high growth rate and biomass production as well as their resistance to a wide range of stress factors (Kleinhenz and Midmore, 2001), and they are well adapted for the wastewater remediation (Arfi et al., 2009). Wastewater from food and farming industries (such as winery (Arfi et al., 2009), animal breeding (Nicholson et al., 1999)) or from other industries (such as printing) can be contaminated by different metals, including copper (Cu), a micro-nutrient that can be toxic at high concentrations and affect the remediation efficiency. Little is known about the absorption and tolerance of metal by bamboos (Collin et al., 2013, 2012). In an hydroponic experiment bamboos, (*Gigantocloa* sp. "Malay dwarf") were shown to tolerate a high Cu concentration (Collin et al., 2013) which is known to be toxic for other Poaceae plants such as wheat (Bravin et al., 2010) and Sabi grass (Kopittke et al., 2009). Therefore, in order to improve phytoremediation technology, it is essential to test the extent of Cu tolerance in bamboos and identify mechanisms by which bamboos are able to cope with an excess Cu content.

Bamboos have been found to be very efficient in accumulating high amounts of silicon (Si) in their tissues, both in natural, i.e. up to 183 mg g^{-1} SiO_2 (Collin et al., 2012), and hydroponic, i.e. up to 218 mg g^{-1} SiO_2 conditions (Collin et al., 2013; Epstein, 1994). There is increasing evidence that Si has a role in alleviating metal toxicity in several species (Guntzer et al., 2012; Liang et al., 2007), and Cu toxicity in *Arabidopsis thaliana* (Khandekar and Leisner, 2011; Li et al., 2008) and in *Erica andevalensis* (Oliva et al., 2011). Although Collin et al. (2013) showed that a wide range of Si in solution (0–1.5 mM) did not influence plant growth and development or the Cu concentration and distribution in *Gigantocloa* sp. bamboos, the absence of Si effect may have been related to the non-toxicity of the Cu concentration tested.

* Corresponding author.
E-mail addresses: collin.blanche@gmail.com, blanche.collin@uky.edu (B. Collin), doelsch@cirad.fr (E. Doelsch), keller@cerege.fr (C. Keller), Patrick.cazevieille@cirad.fr (P. Cazevieille), marie.tella@cirad.fr (M. Tella), chaurand@cerege.fr (P. Chaurand), fredericpanfili@phytorem.com (F. Panfili), jean-louis.hazemann@grenoble.cnrs.fr (J.-L. Hazemann), meunier@cerege.fr (J.-D. Meunier).
[1] Present address: Department of Plant and Soil Sciences, University of Kentucky Agriculture Science Center North, 1100 s. Limestone St., Lexington, KY 40546, USA. Tel.: +1 859 257 2467.

0269-7491/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.envpol.2013.12.024

Bamboo species can be divided into two categories, i.e. monopodial bamboos and sympodial bamboos, according to their rhizome morphology (McClure, 1966). This distinction also reflects their adaptations to the climatic conditions in the native environment: monopodial bamboos are native to temperate climates and sympodial bamboos are native to tropical climates (Kleinhenz and Midmore, 2001). Interestingly, nutrient accumulation differences have been shown between these two bamboo types: monopodial bamboos accumulate significantly more Cu and Si than sympodial bamboos in a similar natural pedoclimatic environment (Collin et al., 2012). In hydroponics, the monopodial bamboo species *Gigantocloa* sp. was shown to tolerate a high Cu concentration (Collin et al., 2013). To optimize the remediation, the "BAMBOU-ASSAINISSEMENT" technology uses several bamboo species from both types. As the extent of Cu tolerance and accumulation might depend on the bamboo species, this should also to be tested in sympodial bamboos.

For the purpose of this study, sympodial bamboos (*Phyllostachys fastuosa*) were grown in a 4-month hydroponic experiment and submitted to two Cu concentrations: an environmentally relevant concentration (Sauvé et al., 1997), and a higher Cu concentration in order to achieve acute toxicity and assess the possible Si-mediated alleviation of metal stress. To identify potential Cu tolerance mechanisms in bamboos, we investigated the distribution and speciation of Cu through an *in situ* multitechnical approach. Characterization (macro- and micro-nutrients, inorganic and organic acids) analyses were first performed on plant samples. Laboratory chemical micro-X-ray fluorescence spectroscopy (μ-XRF) and scanning electron microscopy combined with energy dispersive X-ray analysis (SEM-EDX) were then performed to locate Cu and Si in the root samples. Finally, more detailed insight into Cu speciation and the influence of Si on the local Cu environment was obtained using synchrotron based X-ray absorption spectroscopy, including Cu K-edge X-ray absorption near-edge spectroscopy (XANES) and extended X-ray absorption fine structure spectroscopy (EXAFS).

2. Material and methods

2.1. Silicon and copper treatments

Thirty-five 1-year-old *Phyllostachys fastuosa* bamboos grown on the same substrate were provided by PHYTOREM (Miramas, France). These bamboos were transferred to hydroponic culture after careful washing of the roots in order to remove all soil particles. The experiment was performed over a 4-month period, with a 2-month preculture phase. Details on the hydroponic culture of bamboo, experimental design and preculture nutrient solution composition are given in the hydroponic study of Collin et al. (2013) and in the supporting information (SI 1). At the end of the preculture phase, 25 bamboo plants of uniform size were selected. Five treatments were applied for 70 days, two Si concentrations and three Cu concentrations, with the following combinations: 0 mM Si + 0.1 μM Cu, 0 mM Si + 1.5 μM Cu, 1.1 mM Si + 1.5 μM Cu, 0 mM Si + 100 μM Cu and 1.1 mM Si + 100 μM Cu, hereafter referred to as control, Cu1.5, Cu1.5Si, Cu100 and Cu100Si, respectively. Copper was added in the form of $CuSO_4$. The silicon used was in the form of monosilicic acid from potassium-metasilicate $Si(KOH)_2$ (Metso 400 – YARA) (Voogt and Sonneveld, 2001). Potassium and hydroxide levels were adjusted in the nutrient solution to compensate for the additional input of K and OH from the silicon addition. KNO_3 and HNO_3 concentrations were set at 2.25 mM in each treatment. The macroelement composition (mM) in this nutrient solution was 1 $CaCl_2$, 0.24 $MgSO_4$ and 0.22 $(NH_4)_2HPO_4$ and the microelement composition (μM) was 11.6 H_3BO_3, 0.2 $ZnSO_4$, 0.03 MoO_3 and 6.5 $MnCl_2$. Fe was provided as 20 μM Fe–N, N9-di (2-hydroxybenzyl) ethylenediamine-N, N9-diacetic acid monohydrochloride hydrate (HBED) according to the description of Chaney et al. (1998). The solution pH was set at 6.0 (±0.2), buffered with 1 mM MES (2-morpholinoethanesulphonic acid).

The solution was continuously supplied via a peristaltic pump from a 15-L reserve tank to the base of the five 2.5-L pots, and the solution that exceeded 2.5 L was recovered via an overflow pipe and returned to the reserve tank. The nutrient solutions in each pot were continuously aerated with an air pump. All solutions contained in each pot and in the reserve tank were totally renewed every 7 days. Throughout the experiment, Cu^{2+}, total Cu and total Si concentrations were measured in nutrient solutions—the procedure and the concentration variations during contact with plants are described in the Supporting Information (SI 1). For further details about the variation of Cu and Si uptake between renewal, the reader can refer to the study of Collin et al. (2013).

2.2. Growth parameter measurements and plant sample preparation

The stem number and height, the number of live leaves and fresh weights were assayed at the beginning of the experiment (day 0) during the experiment (day 21 and day 49) and at the end of the experiment (day 226). At the end of the experiment, the 25 plants were harvested. Plant leaves, stems, rhizomes and roots were separated. The samples were carefully washed with ultrapure water and the fresh masses were determined. For each sample, one portion of the material was immediately immersed in liquid N_2 and conserved frozen, and one portion was dried at 60 °C until reaching constant weight. The samples were subsequently mixed, ground and homogenised. The subsamples were dried at 80 °C until they reached a constant weight to determine their dry weight.

2.3. Chemical analysis of macro- and micro-nutrients, anions and amino acids

The dry plant samples (leaves, stems and roots) underwent dry mineralisation to determine the total element concentration. During mineralisation, the Si concentration was determined by gravimetry, as described in Collin et al. (2012). The ash was dissolved in HCl and elemental concentrations were determined using an inductively coupled plasma-optical spectrometer (ICP-OES Vista-Pro, Varian). Cu and Si were analysed in all samples, while N, P, K, Ca, Mg, Fe, Mn and Zn were analysed in the control, Cu100, Cu100Si treatments, in leaf and root samples. For quality control, in-house reference samples and aqueous certified samples (Astasol-Mix, Analytika) were used every 20 samples, and each analysis was conducted in duplicate. The measurement uncertainty was less than 15%. The Si quantification limit was 5 mg g^{-1} of dry weight (DW).

The macro- and micro-nutrient concentrations were expressed as g kg^{-1} or mg kg^{-1} DW, and the Si concentrations were expressed in mg g^{-1} DW SiO_2.

A set of frozen root subsamples was used to measure adsorbed Cu after extraction of apoplasmic Cu with HCl from roots (Chaignon et al., 2002) in the Cu1.5, Cu1.5Si, Cu100 and Cu100Si treatments. Briefly, a subsample of 0.4 g of thawed roots was shaken end-over-end with 20 ml of 1 mM HCl for 3 min, and then with 10 M HCl for 5 min. The suspensions were filtered through ashless filter paper (Whatman 40). Copper in the suspensions was analysed with an inductively coupled plasma mass spectrometer ICP-MS (Q-ICPMS X series II + CCTTM, Thermo Fisher). Root samples were then rinsed thrice with ultrapure water and part of the sample was dried at 80 °C in order to measure the dry weight and the other part was frozen in liquid N_2 to study Cu speciation—these root samples were further referred to as "desorbed roots". The Cu quantities in the suspensions, expressed in μg kg^{-1} DW of roots, were referred to as "Cu adsorbed" in roots.

Anion concentrations were determined in a portion of freeze-dried leaves and roots from the control, Cu100 and Cu100Si treatments by high performance ionic chromatography (HPIC) (Dionex DX 600) using an IonPaq AS11HC anion exchange column and an NaOH gradient. Total soluble amino acids from frozen leaves and roots from the control, Cu100 and Cu100Si treatments were analysed. Amino acids were detected by HPIC (Dionex ICS3000) using a trap column (CRC) and an amino acid column (AminoPac PA10) with an NaOH gradient. Detailed information on these two protocols is given in the Supporting Information (SI 1).

2.4. Laboratory-based μXRF and SEM-EDX

Two different root cross-sections from the Cu100Si treatment were examined using laboratory-based μXRF (HORIBA XGT^{7000}) coupled to an energy dispersive X-ray spectrometer (EDX) and using a Philips XL30 SFEG scanning electron microscope (SEM) at liquid nitrogen temperature coupled to an EDX (See SI 1 for more details).

2.5. EXAFS and XANES: data acquisition and analysis

Copper K-edge X-ray absorption spectra for the plant leaves, stems, roots of treatment Cu100 and Cu100Si, leaves of treatment Cu1.5 and Cu1.5Si, and reference compounds were recorded on the FAME Beamline at the European Synchrotron Radiation Facility (ESRF, Grenoble, France) (Hazemann et al., 2009; Llorens et al., 2012; Proux et al., 2006). In order to avoid artificial speciation changes, frozen leaves, stems and roots were ground and compacted into pressed pellets in liquid N_2 (77 K), with special care taken to keep the pellets frozen in liquid N_2 until the XAS analysis. Pellets of frozen-hydrated plant samples and reference compounds were transferred to a He cryostat and cooled to 15 K to prevent sample damage by photoreduction (Manceau et al., 2002). Spectra were recorded in fluorescence mode using an Si(220) double crystal monochromator and a 30-element solid-state Ge detector (Canberra, France). For each sample, 1–14 scans of 45 min each were averaged. To reduce the risk of beam damage and obtain representative spectra, each scan was focused on a different specimen position. The energy was calibrated using a Cu foil (threshold energy taken at the zero-crossing point of the second derivative spectrum). The data were normalized using Athena software (Ravel and Newville, 2005). We used a combination of principal component analysis (PCA), target transformation (TT), and linear combination fitting (LCF) to fit Cu–K edge XANES spectra (−30–50 keV) and k^3-weighted EXAFS (2.6–10.5 $Å^{-1}$) recorded on the