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Bioindicating potential of strontium contamination with Spanish moss *Tillandsia usneoides*

Guiling Zheng [a], Robert Pemberton [b], Peng Li [a, *]

[a] College of Resource and Environment, Qingdao Agricultural University, Qingdao, 266109, Shandong, China
[b] Florida Museum of Natural History, Gainesville, FL, 32601, USA

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ABSTRACT

Tillandsia species have been recognized as efficient biomonitors of air pollution, but rarely exploited in bioindicating of strontium, an important nuclide. We exposed *Tillandsia usneoides*, colloquially known as Spanish moss due to its filamentous morphology but is an atypical angiosperm in the family Bromeliaceae, to the solutions with different Sr concentrations (0.1–100 mmol/L). The results showed that plants were able to endure Sr stress for a relatively long period, which suggests that *T. usneoides* is able to resist this toxic element. *T. usneoides* had the highest uptake ratio of Sr (82.21 ± 0.12%) when the plants were exposed to 0.1 mmol/L Sr solutions. Sr contents in *T. usneoides* increased significantly with the increase in applied metal solution concentrations. Low Sr stimulated the formation of chlorophyll, but high Sr decreased the contents of chlorophyll, and no significant effect on the total biomass was found in *T. usneoides*. In contrast, the permeability of plasma membrane based on the relative electronic conductivity in *T. usneoides* increased significantly under Sr stress, indicating that Sr probably caused oxidative stress. Moreover, correlation analysis showed that the leaf relative conductivity was significantly positively correlated with Sr contents in the plants after Sr treatments. Therefore, *T. usneoides* has considerable potential for monitoring Sr polluted environments through measuring Sr contents in the plant directly or exploiting the leaf relative conductivity as an indirect biomarker.

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1. Introduction

Radioactive particles can contaminate plants either by foliar deposition and absorption or by uptake from the soil via roots. Foliar contamination might be greater than root contamination during periods with high fallout (Scotti and Carini, 2000). In turn, some plants can be exploited to be suitable indicators of radioactive pollution for their ability to trap and accumulate a variety of nuclides and to retain them for long periods of time. Mosses and lichens have been recognized as suitable biomonitors because they accumulate large amounts of various pollutants, including radionuclides (Conti and Cecchetti, 2001; Onianwa, 2001). The fallout from the Chernobyl accident was assessed, largely, by measuring the concentration of radionuclides in mosses and lichens (Papastefanou et al., 1989; Cevik and Celik, 2009).

Tillandsia usneoides, colloquially known as Spanish moss due to its filamentous morphology, but is not biologically related to either mosses or lichens. Instead, it is an atypical angiosperm in the family Bromeliaceae. Because *T. usneoides* accumulates heavy metals, it has long been proven useful in monitoring air pollution (Calasans and Malm, 1997; Filhoa et al., 2002; Figueiredo et al., 2001, 2007; Pereira et al., 2007a,b; Vianna et al., 2011; Sutton et al., 2014). Many species belonging to *Tillandsia*, including *T. usneoides*, are efficient atmospheric monitors of Hg, Cd, Cr, Pb, Cu, Zn, Co, Ba, V, Mn, Fe, Co, Ni, Cu, Zn, Pb and Br due to their particular physiological features (Brighigna et al., 1997; Malm et al., 1998; Pignata et al., 2002; Wannaz et al., 2006; Bermudez et al., 2009; Rodriguez et al., 2011).

Tillandsia species have the crassulacean acid metabolism that allows their adaptation to extremely dry air and high temperature conditions. They are also epiphytes (without soil penerating roots), and stems and leaves that are covered with highly hygroscopic trichomes that absorb water and nutrients directly from the atmosphere (Benzing, 2000). Therefore, the elemental composition of their tissues and their physiological responses largely reflect the atmospheric input of air pollutants, such as toxic gases and heavy metals (Figueiredo et al., 2001; Pereira et al., 2007a,b; Vianna et al.,

* Corresponding author.
E-mail address: pengleep@163.com (P. Li).

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2011). However, *Tillandsia* species are rarely exploited in nuclides monitoring. Only in the Santiago metropolitan area, Chile, 14.8–63.9 mg/kg Sr and 0.04–0.26 mg/kg Cs were detected by *Tillandsia recurvata* (Cortés, 2004), and in a recent report, *T. usneoides* was found to be have high potential for monitoring Cs, a common radionuclide in the world (Li et al., 2012).

Strontium, also released from nuclear facilities, is an important common radionuclide for the assessment of radiation exposure to the public because of its high fission yield, long half-life (29 years) and transferability (Guillen et al., 2011). Interactions of strontium with environment are comparable with those of calcium because they are alkaline earth metals, and chemical analogs of calcium (Jia et al., 1999). Therefore, it is quite readily available to plants and transferred to the food chain.

Several researchers have previously reported that the fate of radioactive ^{90}Sr in the environment follows the transfer and distribution of stable Sr (Leung and Shang, 2003; Tsukada et al., 2005). The behavior of stable Sr in the environment can be used as a useful analog in predicting the long-term fate of ^{90}Sr. So the present study is directed to 1) evaluate the capacity of Spanish moss *T. usneoides* for uptake and accumulation of stable Sr; 2) identify plant physiological changes (biomass, leaf relative conductivity etc.) resulted from the Sr stress; 3) discuss the potential of *T. usneoides* for bio-indicating stable Sr as an analog of radioactive Sr.

2. Material and methods

2.1. Study species

T. usneoides is native to Southeastern United States and Tropical America, but has been around the horticultural trade from the early 1900s and became a popular indoor ornamental plant worldwide (Benzing, 2000). The plant consists of a slender stem bearing alternate thin and curved leaves, forming chain-like hanging structures. We introduced many strands of *T. usneoides* and cultivated them in the greenhouse. Day/night average temperatures were approximately 27/20 °C. All plants were watered every day by thoroughly wetting all surfaces.

2.2. Sr treatment

Total 15 actively growing plants of *T. usneoides*, each amount of 3 g, were selected for the further experiment. They were divided into 5 groups with each contained 3 replicates. The main aim of the study was to investigate the capability and mechanism of *T. usneoides* Sr uptake. For the sake of convenience and following Wannaz et al. (2011), each plant was soaked in a cup of 500 mL $Sr(NO_3)_2$ solution for two minutes at the same time every day, same as the normal water way. The concentrations of initial $Sr(NO_3)_2$ solutions were 0 mmol/L, 0.1 mmol/L, 1 mmol/L, 10 mmol/L and 100 mmol/L respectively. The treatments ended when the plants treated by the highest Sr solution (100 mmol/L) died because it was impossible to continue further experiments.

After treatment periods, 10 mL solutions were drawn out from the initial solutions and analyzed for Sr concentrations using atomic absorption spectrometry (AAS) (AA700, PerkinElmer Instrument Co. USA). The percentage metal uptake was calculated from % uptake = $[(C_0-C_1)/C_0]*100$, where C_0 and C_1 are initial and remaining concentrations of metal, respectively, in solutions (mg/L).

2.3. Effects of Sr on plants

The plant's leaf morphology, dry weight, leaf chlorophyll content and permeability of plasma membrane were tested as parameters to investigate the effects of Sr stress on *T. usneoides*.

Visible foliar injury of plants was examined and photographed every day. Leaves' color and wilting were recorded as well as the death data of the plant. After treatments, dry weight was tested with 1/1000 balance (Mettler Toledo, Switzerland). Leaves of the plants were harvested and ground into fine powder in liquid nitrogen, and chlorophyll was extracted with 95% ethanol. Chlorophyll content was measured as described previously (Li et al., 2012) and expressed on a dry weight basis. The permeability of plasma membrane was obtained from testing leaf relative electronic conductivity with the conductivity meter (sensION + EC7, USA).

2.4. Sr enrichment in plants

The contents of Sr in the plants of *T. usneoides* were also determined by atomic absorption spectrometry (AAS). Each sample was washed with bi-distilled water, then dried at 70 °C for 10 h. Masses of about 0.5 g of dry material were ground and reduced to ashes. The ashes were digested with $HClO_4$: HNO_3 (1:4) at mild heat and the solid residue was separated by centrifugation. Finally, the volume was increased to 10 mL with bi-distilled water and analyzed by AAS in order to determine the Sr concentrations.

2.5. Data analysis

One-way ANOVA was carried out to compare the difference of means from various treatments and significant statistical differences were established using Tukey's test (SPSS 18.0, IBM, USA). We also calculated the linear correlation coefficient between the Sr contents in the plant leaves and physiological parameters of *T. usneoides*.

3. Results

3.1. Effects of Sr stress on Tillandsia usneoides

Plants of *T. usneoides* treated with the highest Sr concentration (100 mmol/L) were almost completely dry and dead after 14 days. In contrast, *T. usneoides* plants in the 0.1–10 mmol/L treatments remained alive during the treatment period although the stem and leaves turned yellow.

After 14 days of treatment, the total biomass based on the dry weight in 0.1 mmol/L treatment was the highest, which was 0.63 ± 0.04 g, higher than the control. The other three Sr treatments were lower than the control, and the 100 mmol/L treatment was the lowest, which was 0.58 ± 0.02 g (Fig. 1). However, the difference between the total biomasses of *T. usneoides* was not significant in different Sr treatments (One-way ANOVA analysis, $F = 1.308$, $P = 0.332$).

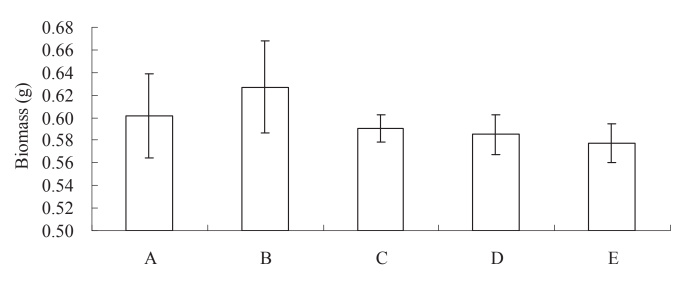

Fig. 1. Effects of ^{88}Sr stress on the total biomass in *Tillandsia usneoides*. Notes: A, 0 mmol/L $Sr(NO_3)_2$; B, 0.1 mmol/L $Sr(NO_3)_2$; C, 1 mmol/L $Sr(NO_3)_2$; D, 10 mmol/L $Sr(NO_3)_2$ and E, 100 mmol/L $Sr(NO_3)_2$.

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