Ecotoxicology and Environmental Safety 97 (2013) 147–153

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Effect of cadmium on the physiological parameters and the subcellular cadmium localization in the potato (*Solanum tuberosum* L.)

Dongyu Xu [a], Zhifan Chen [b], Ke Sun [a], Dong Yan [a], Mingjie Kang [a], Ye Zhao [a,*]

[a] State Key Laboratory of Water Simulation, School of Environment, Beijing Normal University, Beijing 100875, China
[b] College of Environment and Planning, Henan University, Kaifeng 475001, China

ARTICLE INFO

Article history:
Received 9 April 2013
Received in revised form
9 July 2013
Accepted 18 July 2013
Available online 12 August 2013

Keywords:
Cadmium
Physiological parameters
Potato (*Solanum tuberosum* L.)
Subcellular localization

ABSTRACT

The pollution of agricultural soils with cadmium (Cd) has become a serious problem worldwide. The potato (*Solanum tuberosum* L.) was used to investigate how different concentrations of Cd (1, 5, and 25 mg kg^{-1}) affected the physiological parameters and the subcellular distribution of Cd in the potato. The analyses were conducted using scanning electron microscopy coupled with energy dispersive X-ray (SEM-EDX). The results suggest that the leaf is the organ with the highest accumulation of Cd. The malondialdehyde (MDA) content increased and the chlorophyll content decreased in response to high level of Cd. The SEM-EDX microanalysis revealed that Cd was primarily deposited in the spongy and palisade tissues of the leaf. Furthermore, Cd was also detected in the cortex and the adjacent phloem and was observed inside the intercellular space, the interior surface of the plasma membrane, and on the surface of the elliptical starch granules in the tubers of the potato. Although low concentrations of Cd migrated from the root to the tuber, the accumulation of Cd in the tuber exceeded the standard for food security. Therefore, the planting of potato plants in farmland containing Cd should be seriously evaluated because Cd-containing potatoes might present high health risk to humans.

1. Introduction

Heavy metals are ubiquitous in the environment due to the use of metal-containing pesticides and fertilizers in agricultural soils (Liu et al., 2011). If the heavy metals are allowed to accumulate in the crops, toxic metals will pose a threat to human health via the food chain (Sharma and Dietz, 2009; Wagner, 1993). Of all the heavy metals, cadmium (Cd) ranks the highest in terms of damage to plant growth and human health (Gonçalves et al., 2012; John et al., 2008). Cd is easily taken up by plants and translocated to different parts of the plants (Florijn and Vanbeusichem, 1993). This translocation of Cd influences the physiological metabolism of the plant through the displacement of essential cations from specific binding sites, which causes loss of function (Sharma and Dietz, 2009) or the stimulation of the generation of reactive oxygen species (ROS) in plant cells (Noctor et al., 2007), and the inhibition or stimulation of the activity of several antioxidative enzymes (e.g., peroxidase or glutathione reductase) (Chou et al., 2012; Lopez-Huertas et al., 2000). ROS generally stay at acceptable levels under the defense of antioxidant systems and do not cause oxidative damage. However, the over production of ROS might cause oxidative damage, which would result in lipid peroxidation in various plants (Cho and Seo, 2005; Lin et al., 2007). The malondialdehyde (MDA) was routinely used as a general indicator of the extent of lipid peroxidation resulting from oxidative stress (Monteiro et al., 2009). The elevation of MDA indirectly indicates an increase in ROS (Choudhary et al., 2007). Photosynthesis is also sensitive to Cd. Several studies have demonstrated that a reduction in chlorophyll and carotenoid contents should be attributed to the toxicity of Cd (Liu et al., 2011). Therefore, the physiological metabolism parameters (e.g., MDA and/or chlorophyll) could indirectly illustrate the toxicity of Cd in plants.

The subcellular structure of plants plays an important role in the detoxification of the plans in response to the toxicity of heavy metals (Fu et al., 2011; Qiu et al., 2011; Yao et al., 2012). Qiu et al. (2011) indicated that the vacuolar sequestration of leaves should affect the tolerance, detoxification, and hyperaccumulation of Cd and Zn in *Potentilla griffithii*. The subcellular distribution and chemical forms of Cd in *Phytolacca americana* L. was studied by Fu et al. (2011), who suggested that the adaptation of pokeweed to Cd stress should be attributed to Cd compartmentation with organo-ligands in vacuoles or its integration with pectates and proteins in the cell walls of the leaves. In addition, Yao et al. (2012) found that excess Mn in the chloroplast was detoxified by depositing it in a starch granule, which served as a novel detoxifying strategy. These reports all mentioned the importance of the vacuoles of the leaves. In contrast, a number of studies reported the opposite results, such as the absence of Cd in any type of leaf

* Corresponding author. Fax: 86 10 5880 2875.
E-mail address: zhaoye@bnu.edu.cn (Y. Zhao).

0147-6513/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ecoenv.2013.07.021

cell (Katayama et al., 2013) or the temporary binding of Cd^{2+} with PCs in the cytosol (Wojas et al., 2010). Therefore, different species of plants exhibit different characteristics in their response to the stress induced by heavy metals.

The area of Cd-contaminated farmlands reached 1.3×10^3 hm^2 in 25 regions of 11 provinces in China (Sun et al., 2011), which suggests that the crops in this area likely experience Cd toxicity. The potato (*Solanum tuberosum* L.) is rich in starch and protein and is the fourth major crop that contributes to the world's food requirement (Chatterjee et al., 2006). The scientists indicated that the influence of Cd^{2+} on nutrient content in potato was related to the level of Cd^{2+} in the substrate, potato cultivar, plant organ, essential element, growth medium and exposure time (Gonçalves et al., 2009a) . Gonçalves et al. (2009b) also suggested that Cd caused lipid peroxidation in roots and shoot of the two potato cultivars. Furthermore, Gonçalves et al. (2012) found that an association between the long-term diet of potato tuber and a clear anxiolytic effect. Therefore, the fact that potato is exposed to Cd-contaminated farmlands had attracted extensive attention. Moreover, the above-mentioned discovery by Yao et al. (2012) implies that the production of potatoes is facing food security risk because of its high starch contents. Thus, the localization of Cd in the potato and its toxicity should be studied intensively. The objectives of this study were (1) to investigate the impact of different Cd^{2+} on the physiological metabolism parameters of the potato and (2) to analyze the subcellular distribution of Cd in different organs of the potato.

2. Materials and methods

2.1. Pot experiment

The soil (0–30 cm) used in the study was obtained from the TongZhou district of Beijing, China. The soil samples were air-dried at room temperature and then passed through a 2-mm nylon sieve to remove the large debris and biological remnants. The characteristics of the soil were listed in Table S1. The analytical methods used to determine these characteristics were described by Hartley et al. (2009).

A 3 to 3.5 cm of potato (*S. tuberosum* L.) was planted in a plastic pot for 20 days to obtain the seedlings of the potato. In each pot, 2 kg of the soil was contaminated with 0, 1, 5, and 25 mg $kg^{-1}CdCl_2$ solution, respectively. Soluble fertilizers (0.01 percent N, 0.015 percent P_2O_5, and 0.01 percent K_2O) were simultaneously applied to the soils (Lin et al., 2007). All of the soils were incubated for four weeks before the potato was planted. After the incubation, four uniform seedlings of the potato were sown directly into each pot. Each Cd level was analyzed in five replicates. The experiment was conducted under greenhouse conditions.

After the growth cycle of the potatoes, which were harvested and separated into tuber, root, stem, and leaf. The harvestable parts were rinsed with deionized water to remove the soil residue or the dust. The root samples were then immersed in 20 mM EDTA solution for 10 min to remove adsorbed metals on the surfaces and then re-washed with deionized water. All of the separated samples were dried in an oven at 80 °C for 2 days. The dried biomass was ground to pass through a 40 mesh sieve. A total of 200 mg of the sample was digested with HNO_3 and H_2O_2 at 160 °C for 4 h to determine the concentration of Cd. The Cd concentration in the samples was measured using an inductively coupled plasma optical emission spectrometer (ICP-AES; SPECTRO Company, Germany).

2.2. Physiological parameters of the potato leaves

After 70 days of Cd exposure, fresh mature leaves of the potato in each pot were used to analyze the physiological parameters. The MDA content was measured using the method described by Heath and Packer (1968). Briefly, 200 mg of fresh leaves was ground in 0.25 percent thiobarbituric acid (TBA) and 10 percent trichloroacetic acid (TCA) solution. The mixture was then transported into the tubes, which were centrifuged at $10{,}000 \times g$ for 20 min at 4 °C. The supernatant with 5 percent thiobarbituric acid was heated at 95 °C for 30 min in a water bath and then quickly cooled in an ice bath. The heated supernatant was centrifuged at $10{,}000 \times g$ for 10 min. The UV–Visible spectrophotometer (Varian, USA) was used to determine the absorbances of the supernatant at 532 nm and 600 nm. After subtracting the non-specific absorbance (600 nm), the MDA concentrations were calculated using an extinction coefficient of 156 mM cm^{-1} and the following formula (Tang et al., 2010):

$$\text{MDA}(\mu\text{mol MDA g}^{-1}\text{FW}) = [(A_{532} - A_{600})/156] \times 10^3 \times \text{dilution factor} \quad (1)$$

TBA (0.25 percent) in 10 percent TCA was used as the blank.

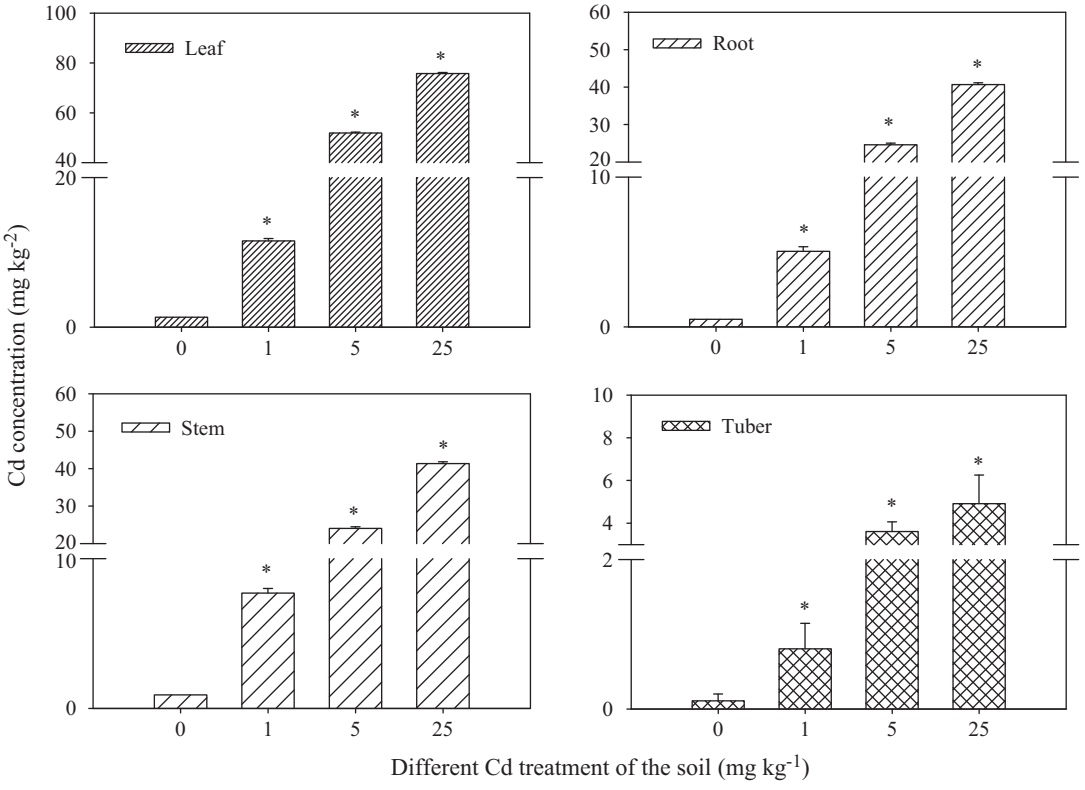

Fig. 1. The accumulation of Cd in the organs of the potato in response to different levels of Cd in the soil. ∗ indicates significant differences at $P < 0.05$ between different concentrations of Cd.