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Effect of humic acid-based amendments with foliar application of Zn and Se on Cd accumulation in tobacco

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A B S T R A C T

The smoke of tobacco is a major source of exposure to Cd in humans and therefore it is urgent to find a way to a method to reduce Cd accumulation in tobacco. A four-month tobacco pot experiment was conducted to investigate the effects of two base treatments (humic acid-based amendments) and two foliar treatments (Zn and Se) on Cd uptake by tobacco. The results showed that Cd in tobacco was mainly transferred into leaves, which could be significantly reduced by both applied amendments. The Cd contents in leaves were reduced by up to 67%. Foliar Zn alone significantly decreased Cd contents in leaves while foliar Se slightly increased them. When base and foliar treatments were combined, base treatments had dominant effects but those of foliar treatments were not distinct. The applied amendments did reduce Cd contents in all the parts of tobacco and the translocation into leaves and they were more effective than foliar Zn and Se.

1. Introduction

Cadmium (Cd) is considered as a highly toxic element without any biological functions for either human or plant. Increasing accumulation of Cd in soil caused by anthropogenic activities will then result in increasing uptake by plants. Human body takes up Cd in food not only through digestive tract but through respiratory tract in tobacco as well. Tobacco (*Nicotiana tabaccum* L.) can greatly bioconcentrate Cd and translocate most of the Cd into leaves which are the direct material for cigarettes. There are more than 300 million smokers in China and cigarettes can basically affect every person because of the numerous second-hand smokers. However, an issue of Cd-contaminated soil has been reported in Hunan Province, one of the main tobacco production places in China, which will probably pose a threat to human health. As a result, it is urgent to find an effective method to reduce Cd accumulation particularly in tobacco leaves.

The amounts of Cd accumulated by plant mainly depend on not only the total contents but also its active fractions (exchangeable fraction for example) in soil (Liang et al., 2014; Nurmesniemi and Poykio, 2006; Zhang et al., 2002). Organic amendments may greatly influence the bioaccessibility of heavy metals by complexation and the transformation of metal into fractions that can hardly be taken up by plants and then reduce metal accumulation in plants (Snyman et al., 2002; Spark et al., 1997).

A majority of studies have found that the effects of exogenous Zn on Cd could be antagonistic, for tobacco (Cherif et al., 2011; Moraghan, 1993; Vasiliadou and Dordas, 2009) and for other plants (Hart et al., 2002, 2005; Lasat et al., 2000; Oliver et al., 1994; Saison et al., 2004). The antagonistic effect was mainly attributed to the similar uptake (Hart et al., 2002; Lasat et al., 2000), transportation mechanisms (Liu et al., 2003), the alleviation of Cd toxicity (Cherif et al., 2011) and enhanced growth and the consequent dilution effect. At the same time, synergistic responses have also been reported as Zn can compete with Cd on phytochelation which helps with sequester Cd in root (Zhu et al., 2003).

Although Se is not considered as an essential element for plants (Lin et al., 2012; Liu et al., 2015), it has been reported that Se at a proper dose could alleviate oxidant stresses caused by heavy metals (Filek et al., 2009; Zembala et al., 2010) and then reduce heavy metal accumulation in plants (Ebbs and Weinstein, 2001; He et al., 2004; Yathavakilla and Caruso, 2007). However, many researches have reported a duel effect (Bluemlein et al., 2009; Cartes et al., 2010; Chen et al., 2008; Fargasova et al., 2006; Ramos et al., 2010) and Se at high doses could also cause stresses, including the damage to the antioxidative system (Cartes et al., 2005; Drake, 2006; Mora et al., 2008).

Most of the published studies focus on either base or foliar treatments but the two methods are seldom researched together. The

Abbreviations: CEC, cation exchange capacity; CK, control check; HA-Ca, humic-calcium; HA-K, humic-potassium; BCF, Bioconcentration Factors; PR, Phytoextraction Rates; TR, Translocation Rates

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aim of this study is to investigate the effect of two base amendments combined with two foliar treatments on the accumulation of Cd in tobacco through pot experiment. The investigation of the Cd translocation from root to shoot in tobacco was also included.

2. Materials and methods

2.1. Plant and soil

Tested soil was collected from a Cd-contaminated area in Hunan Province, China. The properties of tested soil were: total Cd 2.83 mg kg^{-1}, total Zn 108 mg kg^{-1}, pH 4.94, organic matter 48.2 g kg^{-1}, cation exchange capacity (CEC) 22.9 cmol kg^{-1}, total N 2.01 g kg^{-1}, available P 65.8 mg kg^{-1} and available K 69.6 mg kg^{-1}. Tobacco seeds (*Nicotiana tabaccum* L., variety Yunyan 97, provided by Hunan Agricultural University) were fully surface-sterilized in 10% H_2O_2 for 10 min and washed with deionized water thoroughly. Seeds were then germinated with vermiculite in a greenhouse. Tobacco seedlings were transferred into pots after 30 d of germination. Growing conditions were maintained as: 25/20 °C of temperature (day/night), 14 h of photoperiod a day, 70% of relative humidity, 240–350 μmol $(m^2\ s)^{-1}$ of light strength.

2.2. Experimental designs

Each pot was loaded with 2 kg of sieved soil (4 mm). Nine treatments, which were replicated three times, comprised of three base treatments combined with three foliar treatments: blank + foliar water (CK); blank + foliar Zn; blank + foliar Se; humic-potassium (HA-K) + foliar water; HA-K + foliar Zn; HA-K + foliar Se; humic-calcium (HA-Ca) + foliar water; HA-Ca + foliar Zn; HA-Ca + foliar Se.

The humic acid-based amendments, HA-K and HA-Ca (provided by Sino International Ltd.), were added into soil respectively both at the level of 1% by dry soil weight. A blank base treatment with no amendment addition was also included. After the addition of HA-K and HA-Ca, corresponding soil pH were 5.63 and 5.29 (without the plant of tobacco then), respectively. Soil moisture was maintained at 60–70% of water-holding capacity with deionized water. After 60 d of transfer, 0.74 g of $(NH_4)_2PO_4$, 0.73 g of $(NH_2)_2CO$ and 2.78 g of K_2SO_4 were applied as top fertilizers in each pot (K_2SO_4 was not added into soil treated with HA-K, and the amounts of potassium in top applied K_2SO_4 were the same as those in HA-K). After 90 d of the transfer of tobacco seedlings, 14.5 mg of zinc (as $ZnSO_4$) and 1.89 mg of selenium (as Na_2SeO_3) were sprayed evenly and respectively onto leaves of tobacco to make all the leaves wet without dripping. The blank base treatment with foliar treatment of water was considered as the control check (CK). Tobacco plants were harvested after 120 d of transfer, which were then divided into leaves, stems and roots, for the determination of Cd, Zn and Se. The leaves were averagely divided into upper, middle and lower leaves according to the number of leaves of each plant. The leaf number of each plant was generally 20 so there were about 6–7 leaves of each leaf division.

2.3. Determination of Cd, Zn and Se in tobacco

Tobacco samples were washed with deionized water and oven-dried at 105 °C for 30 min and then 75 °C for 48 h. After being dried, samples were ground with a stainless steel mill. 0.250 g of grounded samples were microwave-digested (CEM, MARS5, USA) with 8 mL of HNO_3 at 180 °C for 30 min. Zn and Cd were determined by ICP-MS (Agilent ICP-MS 7700ce, Agilent Technologies, Santa Clara, USA). For Se determination, 4 mL of digestion solutions were added with 1 mL of 6 M HCl and then water-bathed at 98 °C for 2 h. Se concentrations were then determined by fluorescence spectrometer (AFS-920 dual-channel atomic fluorescence spectrometer, Beijing Jitian Instruments Co., Ltd., China). During the whole procedure, digestion blanks and a certified reference material (GSB-27) were included for quality control. For Cd, Zn and Se, the recovery rates of GSB-27 were all among 80–120%. Contents of all the elements and biomasses are presented based on dry weights.

2.4. Bioconcentration Factors (BCF), Phytoextraction Rates (PR) and Translocation Rates (TR) of Cd in tobacco shoots

To evaluate tobacco's potentials for taking up Cd from soil and to translocate it from root to shoot, Bioconcentration Factors (BCF), Phytoextraction Rates (PR) and Translocation Rates (TR) of Cd in shoots were calculated, following Eqs. (1)–(6):

$$A_{soil} = C_{soil} \times W_{soil} \tag{1}$$

$$A_{shoot} = C_{upper} \times W_{upper} + C_{middle} \times W_{middle} + C_{lower} \times W_{lower} + C_{stem} \times W_{stem} \tag{2}$$

$$C_{shoot} = A_{shoot}/(W_{upper} + W_{middle} + W_{lower} + W_{stems}) \tag{3}$$

$$\text{Bioconcentration Factor (BCF)} = C_{shoot}/C_{soil} \tag{4}$$

$$\text{Phytoextraction Rate (PR)} = (A_{shoot}/A_{soil}) \times 100\% \tag{5}$$

$$\text{Translocation Rate (TR)} = C_{shoot}/C_{root} \tag{6}$$

where A represents the amounts of Cd in tobacco shoot and in soil (mg), C is the Cd contents in one particular part of tobacco and in soil (mg kg^{-1}), W refers to the biomasses of one particular part of tobacco and the weight of soil loaded in the pots (kg).

2.5. Statistics analysis

All the data are presented based on dry weights. Analysis of variance was conducted with SAS 9.0 (Least Significance Difference, $p < 0.05$) and figures were drawn with Excel 2010.

3. Results

3.1. Biomass

There was no significant difference among the shoot dry weights under each treatment and that under CK ($p < 0.05$, Table 1). However, both HA-K and HA-Ca alone slightly stimulated tobacco growth, as they increased shoot dry weights by 19% and 31% respectively, compared to that under CK. For foliar treatment alone, Zn elevated shoot dry weight by 32% while Se suppressed that by 21%.

Except for foliar Zn alone, there were no significant differences between the dry weight of each tobacco parts and that under CK either ($p < 0.05$). Similar to shoot dry weights, amendments alone slightly promoted the dry weights of all parts, particularly those of lower leaves and stems. Besides, spray of Zn and Se had contrary effects on the dry weights of all tobacco parts but the effects could be mitigated or reversed when foliar and base treatments were combined (Table 1).

3.2. Cd in tobacco

When applied alone, amendments (without either foliar Zn or Se) significantly decreased the contents of Cd in leaves of tobacco but didn't affect those in roots and stems. Compared to CK (blank with water), both HA-K and HA-Ca significantly reduced Cd accumulation, by up to 67% particularly in middle and lower leaves of tobacco ($p < 0.05$, Fig. 1). HA-K had a greater effect but the difference between the two amendments was not distinct. In roots and stems, Cd contents followed the same order as Blank > HA-Ca > HA-K, but the differences were insignificant. In all parts of tobacco, Cd contents were reduced by 40%, 64%, 67%, 24% and 16% under HA-K and 23%, 50%, 59%, 9% and 11% under HA-Ca respectively, compared to those under CK.