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Effect of calcium carbonate on cadmium and nutrients uptake in tobacco (*Nicotiana tabacum* L.) planted on contaminated soil

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Abstract

In the present study, calcium carbonate ($CaCO_3$) was applied to Cd-contaminated soil at rates of 0, 0.5 and 1.0 g kg^{-1}. The effect of $CaCO_3$ on soil pH, organic matter, available Cd, exchangeable Cd and level of major nutrients in a tobacco field and on accumulation of various elements in tobacco plants was determined. The results showed that $CaCO_3$ application significantly increased the pH level, available P and exchangeable Ca but decreased organic matter, available Cd, exchangeable Cd, available heavy metals (Fe, Mn, Zn and Cu) and available K in soil. Additionally, $CaCO_3$ application substantially reduced Cd accumulation in tobacco roots, stems, upper leaves, middle leaves and lower leaves, with maximum decrease of 22.3%, 32.1%, 24.5%, 22.0% and 18.2%, respectively. There were large increase in total Ca and slight increases in total N and K but decrease to varying degrees in total Fe, Cu and Zn due to $CaCO_3$ application. $CaCO_3$ had little effect on total P and Mn levels in tobacco leaves.

Key words

Bioavailability, Calcium carbonate, Cadmium contaminated soil, Tobacco

Introduction

Hunan province is regarded as "land of non-ferrous metals" in China,. Large areas in Hunan have suffered varying degree of heavy metal pollution, and rice, vegetables and other crops are subject to serious pollution of Cd, As and Pb (Liu *et al.,* 2005; Zhang *et al.,* 2007; Lei *et al.,* 2008; Wang *et al.,* 2008). Hunan is also the major tobacco-producing area in China. In recent years, reports concerning lead (Pb) poisoning incident in Hengyang, the arsenic (As) pollution and poisoning incident in Shimen in Hunan and cadmium (Cd) rice poisoning incident in Hunan have raised great concern about the hazards of heavy metals in cigarettes. Research indicate that tobacco is a high accumulator of Cd; among various parts of tobacco plant, the leaves exhibit highest Cd accumulation capacity (Zhang *et al.,* 2001). A study reported at the 9th Asia-Pacific Conference on Tobacco or Health (2010) showed high level of heavy metals were detectable in 13 brands of cigarettes made in China, among which Cd, As and Pb levels exceeded as much as 3-fold than those cigarettes made in Canada. Tobacco is a product consumed by inhalation, during which heavy metals can enter human body through tobacco smoke. Smoking has become an important source of specific heavy metals for smokers. There is evidence that majority (approximately 70%) of Cd contained in cigarettes enters tobacco smoke, posing a health hazard to both active and passive smokers. The amount of Cd provided by 40 cigarettes per day is equivalent to twice that from food. As compared with other harmful heavy metals, Cd exhibits higher volatility and less adsorption by cigarette filters. Hence, Cd in smoke causes considerable harm to health of smokers (Zeng *et al.,* 2012). After entering human body through smoke, Cd can accumulate to a certain critical level, possibly leading to liver cancer, impaired renal function and other diseases (Klaassen *et al.,* 1999; Patrick, 2003). Therefore, controling Cd accumulation in tobacco is an urgent issue to be addressed. Given the vast tobacco-growing areas with soil heavy metal pollution in China, chemical remediation is economical, effective and fast method as compared to physical and biological remediation. Therefore, use of chemical remediation for heavy metal pollution is more suited to the situation of heavy metal pollution in China. Calcium carbonate ($CaCO_3$) is an affordable and readily available agent. Some studies have shown that soil application of $CaCO_3$ can decrease available heavy metals in soil and attenuate Cd uptake by crops (e.g., rice, vegetables and maize) (Gray *et al.,* 2006; Lee *et al.,* 2009). In the present study, $CaCO_3$ was selected for remediation of soil of tobacco field showing low level of Cd pollution in Guiyang County, Chenzhou City, Hunan Province (China), to reduce Cd uptake and bioaccumulation in tobacco leaves.

Materials and Methods

Characterization of soil and amendments : The experimental field is located in the Wutong Group of Wutong Village in Renyi Town, Guiyang County, Chenzhou City, Hunan Province, China. The study area has a subtropical humid monsoon climate. The pleasant climate exhibits four distinct seasons. The annual average temperature is 17.2°C; annual average sunshine duration is 1,705.4 hrs and annual average rainfall is 1,385.2 mm. A preliminary investigation showed that tobacco fields in the study area were polluted by Cd (Table 1).

Calcium carbonate obtained from commercial company in Zhejiang, was crushed and passed through 80-mesh sieve. Tobacco (*Nicotiana tabacum* L.) seedlings and tobacco-specific fertilizer were provided by the Guiyang Tobacco Company (Chenzhou, China). All of the reagents for sample analysis were of analytical grade.

Field experiments : $CaCO_3$ was thoroughly mixed with soil to obtain homogeneity prior to transplantation of tobacco seedlings. The experimental design included of three treatments with different $CaCO_3$ application rates: 0, 0.5 and 1.0 g kg^{-1} (w/w), and non-amended soil was used as control. Each treatment was independently repeated three times. The plot area was 9 × 9 m for each treatment, and plots were arranged using a randomized block design. Three guard rows were set up around each plot. At maturity, plant height, leaf length and leaf width of tobacco plant were measured and recorded regularly, and plant samples were collected from each plot, including upper leaves, middle leaves, lower leaves, stems and roots. Each plant sample was obtained by mixing single-point samples from five individual plants. The plant samples were then washed with tap water and rinsed with deionized water. Clean samples were wipe-dried and deactivated at 105°C in an oven for 30 min, followed by 75°C heating to constant weight. Dried samples were divided into five parts (upper leaves, middle leaves, lower leaves, stems and roots) and then ground separately using a stainless steel tool. Ground samples were collected to measure total heavy metals, nitrogen (N) and phosphorous (P) in various plant parts.

Table 1: Basic physicochemical properties of the experimental soil

Soil pH	Organic matter (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})	Total K (g kg^{-1})	Total Cd (mg kg^{-1})	Available Cd (mg kg^{-1})
7.85	29.13	0.92	0.39	13.8	19.88	1.95

At the time of sample collection, rhizosphere soil of each plant was collected from roots by gently shaking soil attached to roots. Each rhizosphere soil sample was obtained by mixing five single-point soil samples using same sampling pattern as for the plants. Soil samples were then transported to the laboratory, naturally air-dried and sequentially passed through 100- and 10-mesh nylon sieves. Sieved samples were used to measure the soil pH, organic matter (OM), available Cd, exchangeable Cd, available P, available potassium (K), exchangeable Ca, exchangeable Mg, available zinc (Zn), available copper (Cu), available iron (Fe) and available manganese (Mn) level.

Chemical analysis : Soil pH was measured at water-soil ratio of 2.5:1 using a pHs-3C meter (Shanghai Precision & Scientific Instrument Co., Ltd., China). Soil total N was measured by Kjeldahl distillation method; OM was measured through external heating method with potassium dichromate; available P was measured via sodium bicarbonate extraction / molybdenum blue colorimetry; and available K was measured through ammonium acetate extraction (Simard, 1993). Available heavy metals (Cd, Fe, Mn, Zn and Cu) were extracted using diethylene triamine penta acetic acid (DTPA), and exchangeable fraction Cd was extracted using 1.0 mol l^{-1} $MgCl_2$ (Tessier *et al.,* 1979). Metal concentration in the extracts were assayed by atomic absorption spectrophotometer (Z-2000, Hitachi, Japan). Plant total P and N were digested using concentrated sulfuric acid and hydrogen peroxide at high temperature. Total P in the digestion solution was analyzed via Mo-Sb colorimetry, and total N was determined using Kjeldahl nitrogen analyzer (Simard, 1993). Total heavy metals in plant samples were digested by dry ashing (Zhou *et al.,* 2015), and metal concentration in digestion solution were analyzed by plasma emission spectrometer (ICP 6300, Thermo, USA).

Statistical analysis : Data were expressed as mean ± SD. Data were statistically analyzed via significance F-test and Duncan multiple comparison ($P<0.05$ and $P<0.01$). Statistical analysis was performed using Excel 2010 and SPSS 19.0.

Results and Discussion

It can be observed that with increase in $CaCO_3$ application, there was significant increase in soil pH. The pH values increased gradually from 7.24 to 7.38 and 7.46, increase of 0.14 and 0.22 units, when addition of $CaCO_3$ increased from 0 to 0.5 and 1.0 g kg^{-1}, respectively. Soil OM content exhibited a reverse trend and decreased with increasing $CaCO_3$ application. As compared to control soil (0 g kg^{-1}), addition of $CaCO_3$ increased from 0 to 0.5 and 1.0 g kg^{-1}, OM content reduced by 0.05% and 0.21%, respectively (Fig. 1). However, statistical analysis revealed that increase in pH values were significant under 0.5 and 1.0 g kg^{-1} $CaCO_3$ application ($P<0.05$). Du *et al.* (2009) demonstrated a significant correlation between soil pH and nicotine content. As soil pH varied from 4.0 to 6.5, it was negatively correlated with the nicotine content where as soil pH varied from 6.5 to 9.0, it was positively correlated with nicotine content. In the present study, application of $CaCO_3$ elevated soil pH indicating that it might affect the quality of tobacco leaves. On the other hand, pH increase would inevitably lead to decrease in OM in soil, in agreement with the findings of previous reports. Dai *et al.* (2009) investigated the relationship between surface OM content and pH of soil in China of six geographic regions through statistical analysis. The results revealed significant difference in soil surface pH and OM content among various geographic regions. Soil OM content exhibited a decreasing trend with increasing pH, with these two parameters presenting a highly significant negative correlation ($r = -0.332 – -0.530$, $P<0.001$).

Cd concentrations of DTPA extractable decreased moderately with increasing $CaCO_3$ application rate. As compared with control (0 g kg^{-1}), application of 0.5 and 1.0 g kg^{-1} $CaCO_3$ resulted in decrease in soil available Cd of 0.14 and 0.21 mg kg^{-1}, respectively. However, as compared to control soil, no significant difference ($P>0.05$) was found for Cd concentrations of DTPA extractable as a result of $CaCO_3$ application. Moreover, Cd concentration of exchangeable fraction decreased significantly with increasing rate of $CaCO_3$ application. When $CaCO_3$ application rate reached 0.5 g kg^{-1}, Cd concentration of exchangeable fraction fell below minimum detection limit of the analytical instrument (Fig. 2). Cd concentrations of both DTPA extractable and exchangeable fraction reflects bioavailability of Cd in soil. Decrease in the Cd concentrations of DTPA extractable and exchangeable fraction suggested that $CaCO_3$ application significantly reduced bioavailability of Cd in tobacco field soil. $CaCO_3$ decrease bioavailability of Cd through due to elevation of soil pH increased the amount of negative charges on the surface of soil particles thereby enhancing Cd^{2+} adsorption to soil particles and elevation of soil pH was favorable for Cd^{2+} to form bound precipitates or co-precipitates with hydroxides or carbonates (Lombi *et al.*, 2003; Illera *et al.*, 2004).

Both soil available P and exchangeable Ca gradually increased with increasing rate of $CaCO_3$ application. Application of 0.5 and 1.0 g kg^{-1} $CaCO_3$ resulted in increases in soil available P by 18.4% and 52.2%, and increase in exchangeable Ca by 5.1% and 10.2%, respectively, as compared with that control (0 g kg^{-1}). Statistical analysis showed that significant difference occurred in 1.0 g kg^{-1} $CaCO_3$ treatments as compared with control ($P<0.05$). While soil available K and exchangeable Mg showed no significant changes (Table 2) with increases in $CaCO_3$ application rate as compared with control ($P<0.05$). The possible mechanism through which $CaCO_3$ increases available P is that $CaCO_3$ elevates soil pH, and thus, phosphate adsorbed on soil particles is substituted and released. Increase in

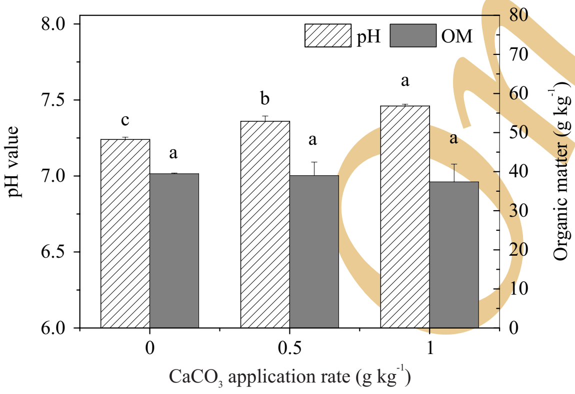

Fig. 1 : Effect of $CaCO_3$ on soil pH and organic matter contents (Error bars indicate standard errors of 3 replicates. The different letters indicate significant difference at $P<0.05$, similarly hereinafter)

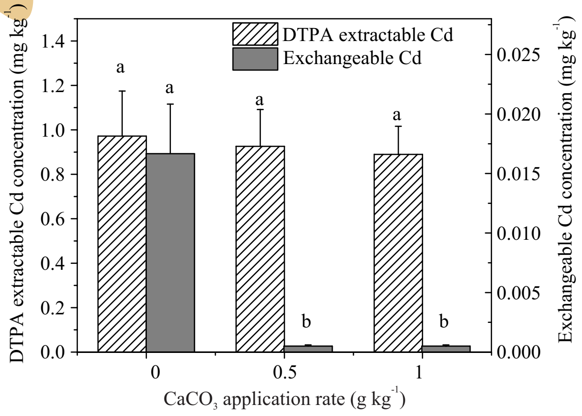

Fig. 2 : Effect of $CaCO_3$ on the concentrations of available Cd and exchangeable Cd in tobacco field soil

exchangeable Ca was expected to be due to direct effect of $CaCO_3$ application. Regarding decrease in available K, $CaCO_3$ acts through same mechanism associated with decrease in bioavailability of Cd.

With increase in $CaCO_3$ application, soil available Fe, Mn, Cu and Zn displayed decrease to varying degrees. As compared with control, 0.5-1.0 g kg^{-1} $CaCO_3$ application resulted in decrease in available Fe, Cu, Zn and Mn by 5.7-13.6%, 6.1-13.7%, 3.3-11.0%, and 0-0.85%, respectively. However, soil available Fe, Zn and Mn showed no significant ($P>0.05$) change, excepted available Cu under 1.0 g kg^{-1} $CaCO_3$ application (Table 3). Decreases in soil available Fe, Cu and Zn could be attributed to increase in soil pH induced by $CaCO_3$.

Cd concentration in various plant parts was found in following order: lower leaves > middle leaves > upper leaves > roots > stems, indicating high capacity of tobacco for Cd accumulation (Table 4). These findings is in agreement with the results of previous studies (Frank, 1985; Cao *et al.,* 2015). Moreover, Cd concentration in various parts of tobacco plants showed certain decrease with increase in $CaCO_3$ application. As compared with control (0 g kg^{-1}), application of 0.5-1.0 g kg^{-1} $CaCO_3$ resulted in Cd concentration of roots and stems decreased by 11.2-22.3% and 17.8-32.1%, respectively. In tobacco leaves, application of 0.5-1.0 g kg^{-1} $CaCO_3$ resulted in reduction in Cd content by 12.6-24.5% in upper leaves, 5.7-22.0% in middle leaves and 8.1-18.2% for lower leaves, respectively. Excepted upper leaves and middle leaves, there were significant differences occurred in 1.0 g kg^{-1} $CaCO_3$ treatments as compared with control ($P<0.05$). Thus, $CaCO_3$ application clearly reduced Cd uptake and translocation in tobacco plants. The primary mechanism through which $CaCO_3$ exerts this function might be due to increase in soil pH induced by $CaCO_3$, which in turn reduced bioavailability of Cd in soil (Fig. 2). Zeng *et al.* (2012) applied Ca-Mg-P fertilizer and sepiolite to Cd-polluted soil in tobacco field and found that both amendments were able to reduce Cd concentration in various parts of tobacco plants. These authors proposed that elevation of soil pH by Ca-Mg-P fertilizer and sepiolite was one of the main cause of inhibition of Cd accumulation in tobacco. Studies have shown that Ca^{2+} competes with Cd^{2+} for absorption sites in plant roots (Andersson and Nilsson, 1974) and suppresses Cd toxicity in plants (Österås and Greger, 2003). The amendment of soil with Ca-containing substances significantly alleviates Cd toxicity, not only in appearance but also in biomass. Moreover, this strategy can significantly reduce Cd concentrations in the aboveground parts of maize (Wang *et al.,* 2001). Kim *et al.* (2002) showed that Cd can be absorbed by plants through competition with Ca for plasma membrane transporters. Therefore, amendment with Ca reduces Cd accumulation in plants mainly through the competitive absorption of Ca and Cd by plants. However, Song *et al.* (2009) reported that amendment of polluted soil with Ca resulted in higher Cd concentration in soil solution but lower Cd concentration in small rape as compared with that of control.

Table 2 : Effect of $CaCO_3$ on the concentrations of available P and K and exchangeable Ca and Mg in tobacco field soil

$CaCO_3$ application rate (g kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})	Exchangeable Ca (cmol kg^{-1}) 1/2 Ca^{2+}	Exchangeable Mg (cmol kg^{-1}) 1/2 Mg^{2+}
0	87.36±5.89b	126.07±8.57a	97.69±3.54b	1.68±0.10a
0.5	103.41±31.41ab	118.35±9.34a	102.65±3.93ab	1.65±0.51a
1.0	132.96±41.14a	119.71±18.81a	107.67±4.10a	1.67±0.03a

Table 3: Effect of $CaCO_3$ on the concentrations of available Fe, Cu, Zn, and Mn in tobacco field soil

$CaCO_3$ application rate (g kg^{-1})	Available Fe (mg kg^{-1})	Available Cu (mg kg^{-1})	Available Zn	Available Mn (mg kg^{-1})
0	65.76±7.02a	62.45±4.67a	63.03±5.13a	25.46±1.81a
0.5	61.98±5.97a	58.67±3.73ab	60.98±5.04a	26.36±4.70a
1.0	56.82±4.31a	53.91±4.94b	56.07±4.12a	24.61±1.71a

Table 4: Effect of $CaCO_3$ on Cd concentrations in various tissues of tobacco plants

$CaCO_3$ application rate (g kg^{-1})	Root (mg kg^{-1})	Stem (mg kg^{-1})	Leaf (mg kg^{-1})		
			Upper leaves	Middle leaves	Lower leaves
0	4.32±0.42a	1.70±0.27a	15.27±2.77a	16.79±1.81a	18.12±1.62a
0.5	3.84±0.65ab	1.40±0.24ab	13.35±1.50a	15.83±0.50a	16.65±0.47ab
1.0	3.36±0.51b	1.16±0.22b	11.54±1.47a	13.09±1.03a	14.81±1.71b

Table 5: Effect of $CaCO_3$ on N, P, K, Ca and Mg levels in the middle leaves of tobacco plants

$CaCO_3$ application rate (g kg^{-1})	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (mg kg^{-1})
0	11.51±0.83b	2.84±0.60a	39.67±1.21a	21.19±0.74a	30.91±1.85a
0.5	13.05±2.11a	2.71±0.50a	41.72±0.43a	22.21±1.55a	30.23±4.14a
1.0	12.92±0.22ab	2.82±0.51a	41.91±2.85a	23.47±1.61a	31.85±0.70a

Table 6 : Effect of $CaCO_3$ on Fe, Cu, Zn and Mn concentrations in the middle leaves of tobacco plants

$CaCO_3$ application rate (g kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})
0	28.92±8.08a	18.83±4.07a	67.18±8.02a	35.31±5.43a
0.5	25.81±7.26a	17.04±1.45a	62.83±2.46a	36.22±4.59a
1.0	14.03±4.24b	16.02±0.99a	59.47±3.59a	33.77±1.55a

With increase in $CaCO_3$ application, there were slight increase in leaf N and K content and large increase in Ca concentration, whereas no significant (P>0.05) changes in P, K, Ca, and Mg were observed. As compared with control, 0.5-1.0 g kg^{-1} $CaCO_3$ application resulted in increase in leaf N, K, and Ca of 13.0%-12.2%, 5.3%-5.8%, and 4.8%-10.8% respectively, and a significant difference was observed in 1.0 g kg^{-1} $CaCO_3$ treatment ($P<0.05$) in leaf N concentration (Table 5). Research indicate that excessively high or low concentration of N and P are unfavorable for tobacco leaves. There was generally a positive correlation between N and nicotine content in tobacco leaves. Higher nicotine content results in improved strength, enhanced irritation and a spicy flavor, while tobacco leaves with lower nicotine contents exhibit low strength and a flat flavor (zhang *et al.*, 2001; Zeng *et al.,* 2012). P deficiency negatively affect the aroma and taste of tobacco leaves, whereas high P concentration results in thick, brittle leaves with poor oil content and stiffness after curing. In the present study, $CaCO_3$ application had little effect on leaf N and P in tobacco. K and Ca were major components of ash in tobacco leaves. K and Ca in tobacco leaves supported combustion, and leaves with high K concentration were relatively soft and flexible. An appropriate level of Mg contributes to combustibility of tobacco leaves and causes leaves to appear grayish white. Moreover, Mg has a coagulating effect on tobacco ash and makes it difficult to scatter. This effect of Mg played a positive role in improving the quality of cigarettes especially cigars (Liu and Zhao, 2009). In the present study, application of $CaCO_3$ increased total K, Ca and Mg concentration in tobacco leaves, thus playing a positive role in improving the quality of tobacco.

With increasing $CaCO_3$ application, leaf Fe, Cu, and Zn exhibited decrease to varying degrees (Table 6). As compared with control, 0.5-1.0 g kg^{-1} $CaCO_3$ application resulted in Fe, Cu, and Zn concentration in leaves decrease by 10.8-51.5%, 9.5-14.9%, and 6.5-11.5%, respectively; and significant ($P<0.05$) change occurred in leaf Fe (1.0 g kg^{-1}). Few studies have investigated the effect of leaf Fe, Cu, Mn, and Zn concentration on the quality of tobacco. Nevertheless, an appropriate level of these elements in tobacco leaves guarantees normal growth in tobacco plants and high quality cigarettes (Jie *et al.,* 2010).

$CaCO_3$ application significantly increased the pH level but slightly decreased OM contents in soil of tobacco field. $CaCO_3$ application substantially reduced Cd concentrations of bioavailable in soil, thus decreasing Cd level in tobacco roots, stems and leaves. Therefore, $CaCO_3$ can be used to remediate Cd pollution in tobacco fields and alleviate Cd accumulation in tobacco leaves, ultimately reducing the toxic effect of tobacco leaves to human health. $CaCO_3$ inhibited Cd uptake and translocation in tobacco plants, and this amendment also had a significant effect on nutrient uptake from soil by tobacco plants. $CaCO_3$ application resulted in higher concentration of available P and exchangeable Ca but lower concentration of available Fe, Cu, and Zn in soil. No significant changes in soil available K, exchangeable Mg, and available Mn or in leaves P, Mg, and Mn were observed. Tobacco leaves exhibited slight increase in N and K content and a large increase in Ca due to $CaCO_3$ application; whereas Fe, Cu, and Zn level decreased to varying degrees. $CaCO_3$ application had little effect on total P and Mn in tobacco leaves.

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