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Effect of 2,4,6-trimercaptotriazine, trisodium salt, nonahydrate on heavy metals bioavailability in soils and accumulation in tobacco

*Yansong Xiao[1], Qiulong Hu[2], Hongguang Li[1], Quan Zhong[1], Wei-ai Zeng[3], Songsong Gu[2], Hailin Cai[3], Lin Tan[2]**

[1]Chenzhou Tobacco Company of Hunan Province, Chenzhou, Hunan, P.R. China
[2]Hunan Agricultural University, Changsha, Hunan, P.R. China
[3]Changsha Tobacco Company of Hunan Province, Changsha, Hunan, P.R. China
**Corresponding author: hqltanlin@163.com*

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Abstract: Pot experiments were carried out to study the effects of a novel stabilizer of 2,4,6-trimercaptotriazine, trisodium salt, nonahydrate (TMT) on the bioavailability of heavy metals (Cu, Zn, As, Cd and Pb) in soils and heavy metals accumulation in tobacco. The results showed the optimal TMT dosage 200 mL/kg could effectively reduce the bioavailability of heavy metals in soils, and the bioavailability of Cu, Zn, As, Cd and Pb in soils was reduced simultaneously by up to 73.1, 63.2, 48.0, 68.9 and 57.2%, respectively. Application of TMT could significantly decrease Cu, Zn, As, Cd and Pb contents of all parts of tobacco. Furthermore, the contents of available Cu, Zn, As, Cd and Pb in soils were significantly positively correlated with Cu, Zn, As, Cd and Pb contents in upper, middle and lower leaves and stems of tobacco. There were no significant differences among plant height, number of leaves, stem girth, length and width of maximum leaves of tobacco under TMT treatments and that under control ($P < 0.05$), which showed that the TMT did not promote and inhibit the growth of tobacco.

Keywords: toxic elements; *Nicotiana tabacum* L.; available contents; correlation; agronomic traits

A study reported at the Ninth Asia-Pacific Conference on Tobacco or Health (2010) showed that high levels 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 the same cigarettes in Canada. Comparative evaluation and toxicity assessment of heavy metals (Pb, Cd, Ni, Cr and Zn) in commonly smoked cigarette brands and local tobacco snuff purchased and consumed in Nigeria were reported (Vincent et al. 2011). The levels of toxic metals (Cd, Cu, Co, Ni, Zn and Pb) in the tobacco of different Iranian cigarette brands and related health issues were investigated (Pourkhabbaz and Pourkhabbaz 2012). An assessment was conducted to determine that the levels of Cd, Pb, Cu and Zn in cigarettes sold in Ethiopia compare well with levels of cigarettes from other parts of the world (Engida and Chandravanshi 2017). These reports not only raised the public cigarette security concerns but also caused the research of heavy metals in tobacco (Zeng et al. 2016).

In situ immobilization of heavy metals by using extraneous active amendments has been considered as high efficiency and low-risk remediation measure for contaminated soils. Some immobilization amendments were applied into the heavy metals contaminated soils, such as limestone, zeolite, phosphate and organic material in recent years, as well as nano-bio-repair materials and their combinations (Cao et al. 2015, Hu et al. 2018, Liu et al. 2018, Xiong et al. 2018, Zeng et al. 2018). Possible mechanisms for soil immobilization mainly include adsorption,

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precipitation, co-precipitation and chelation (Basta and McGowen 2014, Bolan et al. 2014), and thereby decrease the bioavailable fractions of heavy metals or change their redox states. Thus, the effectively decrease the mobility, bioavailability and toxicity of the heavy metals in soils. Therefore, the method of in situ immobilization remediation of heavy metals-contaminated soils has been widely used. However, only a few studies focused on the simultaneous immobilization of Cu, Zn, As, Cd and Pb in tobacco planting soils.

2,4,6-trimercaptotriazine, trisodium salt, nonahydrate (TMT) is an organic sulfur compound. The special sulfur group of TMT can combine with heavy metals by chelate precipitation (Henke et al. 2000); TMT has thus been widely used in the treatments of wastewater and smelter-contaminated soil containing heavy metals (Matlock et al. 2001, Jiang and Zhou 2018). However, studies on the application of TMT for simultaneous immobilization of Cu, Zn, Pb, Cd and As in soils and for a decrease of heavy metals contents in tobacco were rarely conducted. In this study, tobacco pot experiments were carried out to study the effects of a novel stabilizer of 2,4,6-trimercaptotriazine, trisodium salt, nonahydrate on the bioavailability of heavy metals (Cu, Zn, As, Cd and Pb) in soils and heavy metals accumulation in tobacco. The results of this study will contribute to an effective and potential method for simultaneous control of heavy metals bioavailability in tobacco planting soils, and provide a theoretical basis and technical support for the reduction of heavy metals accumulation in tobacco.

MATERIAL AND METHODS

Materials. The tested tobacco cultivar was Yunyan 87 from Hunan Provincial Tobacco Company, Changsha City, China. The tested soils were rotation soil (0–20 cm) for tobacco and rice from the Hunan Agricultural University Base of the Middle South Agricultural Experimental Station of China Tobacco, and the soil type was yellow soil. The collected soils were air-dried, sieved using a 2 mm sieve and placed into plastic bags for property analysis. The soil physical and chemical properties were measured by a previously reported method (Lu 1999) and the results were as follows: pH 6.08, total organic carbon 17.1 g/kg, alkali-hydrolyzable N 88.35 mg/kg, available P 18.68 mg/kg, available K 93.2 mg/kg, exchange capacities (CEC) 121.2 $mmol_+$/kg. The TMT solution (mass concentration 60%) were purchased from the Suzhou Rundong Environmental Protection and Technology Co., Ltd in China.

Pot test. Pot experiments were carried out in the greenhouse of the College of Plant Protection, Hunan Agricultural University from March 26 to June 16, 2017. The tested soil samples (10.0 kg) were added into a plastic bucket and mixed with the TMT diluents at room temperature, and the TMT dosage reached 50, 100, 150, 200 and 250 mL/kg soil. Soil without any treatment was considered as the control (CK). Therefore, there were a total of 6 treatments. Three pots were used for each treatment.

The soils were then treated with tobacco active organic and inorganic base fertilizer (total nutrient ≥ 29%, the ratio of nitrogen, phosphorus and potassium = 8:10:11) at room temperature for a week before transplanting tobacco seedlings. Two seedlings were transplanted (at 2 cm depth) into each pot. During the growing stage, special tobacco fertilizer (total nutrient content ≥ 42%, the ratio of nitrogen, phosphorus and potassium is 10:0:32) and agricultural potassium nitrate (total nutrient = 58%, total potassium ≥ 44.5% and total nitrogen (N) ≥ 13.5%) were applied once. At the maturation stage, the upper, middle and lower leaves and stems of 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 to constant weight, samples were ground with a stainless steel mill. The soil samples of each plant were collected from roots by shaking soil attached to roots. The soil samples were then transported to the laboratory, naturally air-dried and sequentially sieved using a 100-mesh sieve, and collected for future measurement.

Analysis method for total concentrations and bioavailability of Cu, Zn, Pb, Cd and As in soils. The total concentrations of Cu, Zn, Pb, Cd and As were determined by ICP-MS (Thermo Fisher Scientific, USA). The available Cu, Zn, Pb and Cd contents in tobacco planting soils were evaluated by the extracting agent diethylenetriaminepentaacetic acid (DTPA) (NY/T 890-2004, GB/T 23739-2009) and were analysed by ICP-MS. The available As content in soils was evaluated by the extracting agent $NaHCO_3$ (Woolson et al. 1971) and was analysed by ICP-MS.

Analysis method for Cu, Zn, Pb, Cd and As contents of tobacco. Tobacco samples (0.2 g, weighed accurately to 0.0001 g) were microwave-digested (CEM, Matthews, USA) with 8 mL of HNO_3 at 180°C for 30 min. Cu, Zn, Pb, Cd and As concentration in digestion solution were determined by ICP-MS (Thermo Fisher Scientific, Rochester, USA)

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Table 1. Heavy metals contents of tested soils (mg/kg)

	Cu	Zn	As	Cd	Pb
Tested soils	25.22	80.17	20.53	0.38	33.59
Risk intervention values for soil contamination for agricultural land in China (GB 15618-2018)	50	200	40	0.30	90

Data processing and analysis. All data were measured three times and analysed using Microsoft Excel 2003 software (Redmond, USA). Statistical analyses were performed using SPSS 17.0 statistical software (Armonk, USA).

RESULTS AND DISCUSSION

Effects of TMT dosage on the bioavailability of heavy metals Cu, Zn, Pb, Cd and As in soils. The accumulation of heavy metals by plants depends not only on the total content but mainly on the active fractions, including the most active fraction, bioavailable metals in soils (Nurmesniemi and Poykio 2006, Zhang et al. 2013, Liang et al. 2014). In the present study, DTPA-extractable Cu, Zn, Cd and Pb and $NaHCO_3$-extractable As were recognized as predictors of Cu, Zn, As, Cd and Pb bioavailability, respectively (Dai et al. 2004, Arco-Lázaro et al. 2017). Thus, the effects of TMT dosage on the available contents of heavy metals Cu, Zn, As, Cd and Pb in tobacco-planting soils were investigated.

Table 1 showed the chemical properties of initial soils. Figure 1 showed the influence of TMT dosage on the heavy metals Cu, Zn, Pb, Cd and As bioavailability in rhizosphere soils of tobacco after 80 days of growth. The DTPA-extractable concentrations of Cu, Cd and Pb decreased with increasing TMT dosage in the range of 50 mL/kg to 200 mL/kg and then tended to be stable at the TMT dosage of 200 mL/kg and 250 mL/kg. The DTPA-extractable concentrations of Cu, Cd and Pb decreased to 0.815, 0.081 and 0.98 mg/kg at a TMT dosage of 200 mL/kg, respectively. Compared with the control treatment, the immobilization efficiencies of Cu, Cd and Pb at a TMT dosage of 200 mL/kg reached 73.1, 68.9 and 57.2%, respectively. Compared with the control treatment, the immobilization efficiencies of Zn and As reached 63.2% and 48.0%, respectively, at a TMT dosage of 200 mL/kg and then decreased slightly with increasing TMT dosage of 250 mL/kg. Therefore, the optimal TMT dosage was 200 mL/kg to immobilize Cu, Zn, Pb, Cd and As simultaneously in tobacco-planting soils.

Effects of TMT dosage on Cu, Zn, As, Cd and Pb accumulation in tobacco. Figure 2 showed the distribution of Cu, Zn, As, Cd and Pb in different parts of tobacco. The order of Cu content in tobacco is upper leaves > stems > middle leaves > lower leaves; the order of Zn content in tobacco is upper leaves > middle leaves > lower leaves > stems; the order of As, Cd and Pb contents in tobacco are lower leaves > middle leaves > upper leaves > stems.

Figure 2a showed the effects of TMT dosage on Cu, Zn, As, Cd and Pb accumulation in upper leaves of tobacco. Compared with the control treatment, application of TMT in the dosage range of 50 mL/kg to 250 mL/kg reduced Cu, Zn, As, Cd and Pb contents by 23.14–53.49, 18.80–44.27, 8.5–22.76, 14.63–36.24, 13.67–29.86%, respectively. Figure 2b showed the effects of TMT dosage on Cu, Zn, As, Cd and Pb accumulation in middle leaves of tobacco. Compared with the control treatment, application of TMT in the dosage range of 50 mL/kg to 250 mL/kg reduced Cu, Zn, As, Cd and Pb contents by 17.74–45.94, 15.05–35.58, 10.19–28.03, 14.63–43.77, 10.46–26.76%, respectively. Figure 2c showed the effects of TMT dosage on Cu, Zn, As, Cd and Pb accumulation in lower leaves of tobacco. Compared

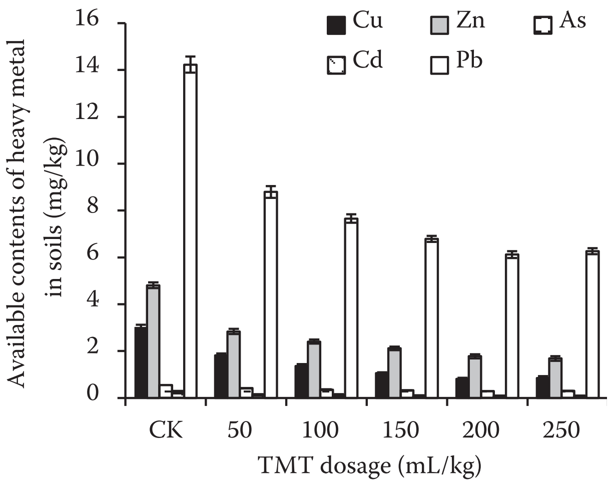

Figure 1. Influence of 2,4,6-trimercaptotriazine, trisodium salt, nonahydrate (TMT) dosage on the bioavailability of heavy metals Cu, Zn, As, Cd and Pb in soils. CK – control

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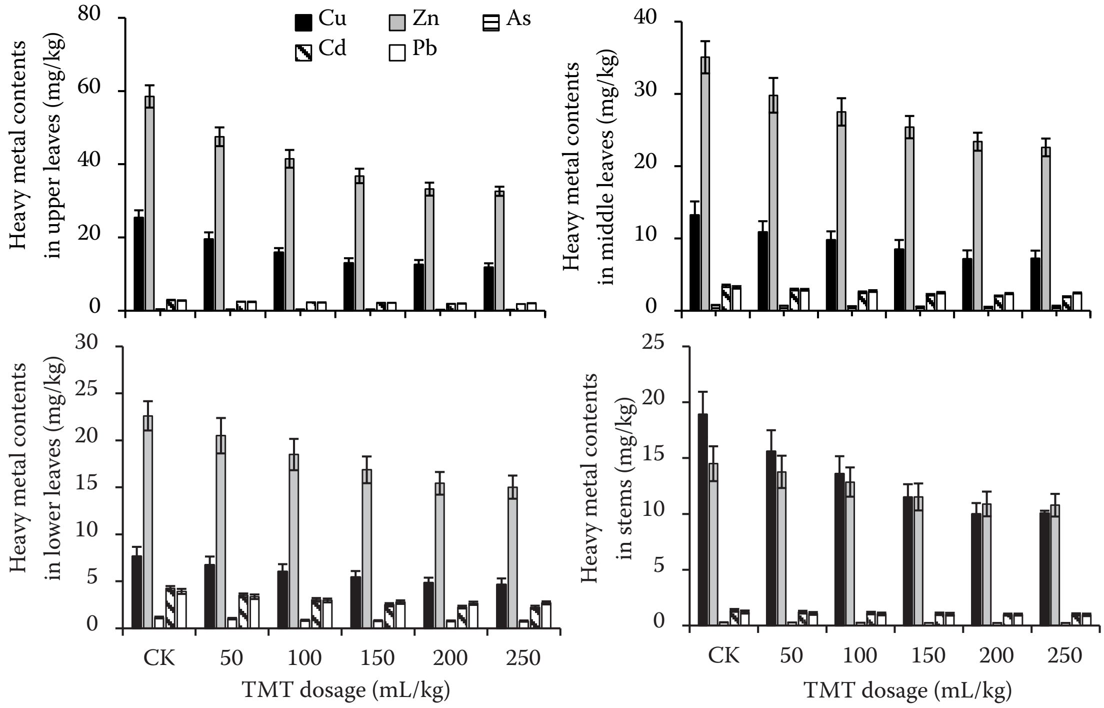

Figure 2. Effects of 2,4,6-trimercaptotriazine, trisodium salt, nonahydrate (TMT) dosage on heavy metals contents in various tissues of tobacco plants. CK – control

with the control treatment, application of TMT in the dosage range of 50 mL/kg to 250 mL/kg reduced Cu, Zn, As, Cd and Pb contents by 11.76–39.08, 9.29–33.548, 9.89–32.16, 17.64–47.76, 14.79–31.12%, respectively. Figure 2d showed the effects of TMT dosage on Cu, Zn, As, Cd and Pb accumulation in stems of tobacco. Compared with the control treatment, application of TMT in the dosage range of 50 mL/kg to 250 mL/kg reduced Cu, Zn, As, Cd and Pb contents by 17.46–47.20, 11.22–30.45, 5.17–27.47, 11.11–43.77, 10.74–21.49%, respectively.

Based on the above data, the Cu, Zn, As, Cd and Pb contents in different parts of tobacco decreased with increasing TMT dosage in the range of 50 mL/kg to 200 mL/kg, and then remained stable or decreased slightly with increasing TMT dosage at the TMT dosage from 200 to 250 mL/kg. The results showed that application of TMT could effectively simultaneously reduce Cu, Zn, As, Cd and Pb contents of all parts of tobacco.

Correlation of bioavailability of heavy metals in soils with heavy metals contents in different parts of tobacco. The correlations between bioavailability of heavy metals in soils with different treatments and heavy metals contents in different parts of tobacco were analysed. The results showed that the contents of available Cu, Zn, As, Cd and Pb in soils were significantly positively correlated with Cu (Figure 3a), Zn (Figure 3b), As (Figure 3c), Cd (Figure 3d) and Pb (Figure 3e) contents in upper, middle and lower leaves and stems of tobacco, all R^2 values exceeded to 0.809, the maximum R^2 value was 0.981 ($P < 0.05$), which were consistent with the results in the former researches (Yu et al. 2010, Chen 2014, Zhu et al. 2015).

Effects of TMT dosage on agronomic traits of tobacco. The agronomic traits of tobacco with the different TMT dosage treatments were presented in Table 2. There were no significant differences among plant height, a number of leaves, stem girth, length and width of maximum leaves of tobacco under different treatments and that under CK ($P < 0.05$, Table 1). The results showed that the TMT did not promote and inhibit the growth of tobacco.

In conclusion, to reduce the heavy metals Cu, Zn, As, Cd and Pb simultaneously in tobacco, TMT was used as the chemical amendment for tobacco planting soils. The main conclusions were as follows:

The bioavailability of Cu, Zn, As, Cd and Pb in soils were reduced simultaneously by up to 73.1, 63.2, 48.0, 68.9 and 57.2% at the optimal TMT dosage of 200 mL/kg. Application of TMT could significantly decrease Cu, Zn, As, Cd and Pb contents of all parts of tobacco. The contents of available Cu, Zn, As, Cd and Pb in soils were significantly positively correlated

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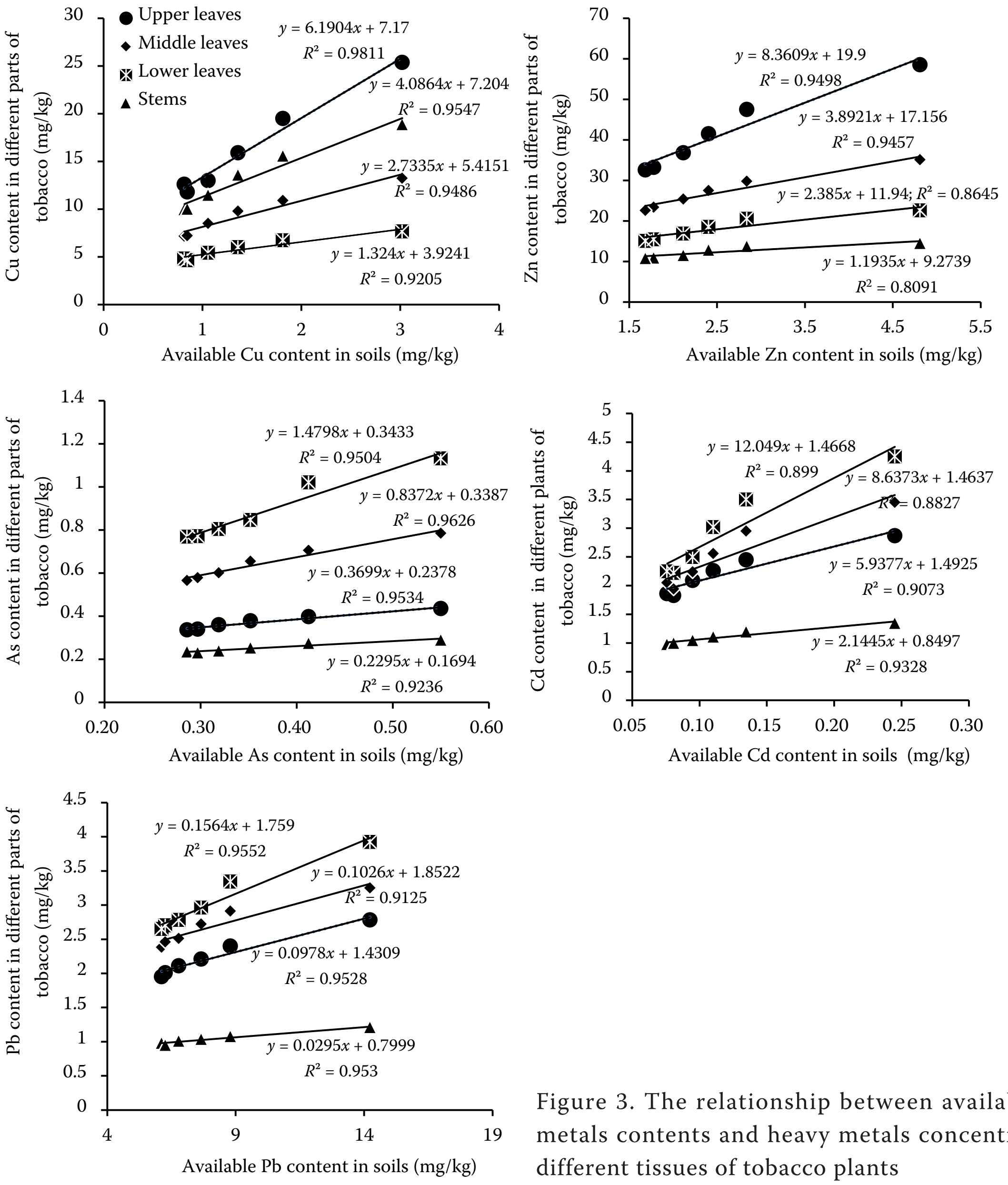

Figure 3. The relationship between available heavy metals contents and heavy metals concentrations in different tissues of tobacco plants

Table 2. Effects of 2,4,6-trimercaptotriazine, trisodium salt, nonahydrate (TMT) treatments on agronomic traits of tobacco

TMT dosage (mL/kg)	Plant height (cm)	Number of leaves (pieces)	Stem girth	Length of maximum leaves	The width of maximum leaves
				(cm)	
CK	93.83 ± 2.42^a	18.67 ± 0.58^a	6.74 ± 0.05^a	63.57 ± 1.82^a	24.85 ± 1.23^a
50	89.87 ± 2.23^a	19.33 ± 1.15^a	7.02 ± 0.08^a	61.67 ± 1.75^a	25.77 ± 1.10^a
100	94.52 ± 2.12^a	18.33 ± 0.58^a	6.52 ± 0.07^a	65.41 ± 1.80^a	23.89 ± 1.05^a
150	87.93 ± 1.98^a	19.00 ± 1.00^a	6.34 ± 0.07^a	66.83 ± 1.62^a	24.24 ± 1.18^a
200	91.74 ± 2.75^a	19.33 ± 1.15^a	6.87 ± 0.09^a	63.24 ± 1.73^a	25.21 ± 1.32^a
250	92.42 ± 2.99^a	18.67 ± 0.58^a	6.59 ± 0.05^a	66.52 ± 1.54^a	24.91 ± 1.25^a

Data are reported as the mean value plus or minus standard deviation (n = 3). The same letters indicate no significant differences among agronomic traits of tobacco under different treatments (P < 0.05). CK – control

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with Cu, Zn, As, Cd and Pb contents in upper, middle and lower leaves and stems of tobacco. Through comparison of agronomic traits of tobacco, there were no significant differences under different TMT treatments and that under CK ($P < 0.05$). Therefore, it is potentially effective to reduce the bioavailability of heavy metals (Cu, Zn, As, Cd and Pb) in tobacco planting soils and reduce the absorption of heavy metals in tobacco simultaneously using TMT.

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