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The influence of humic acids on the accumulation of lead (Pb) and cadmium (Cd) in tobacco leaves grown in different soils

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Abstract:

Four types of soil (red, yellow, cinnamon, and paddy soil) from China were used to conduct experiments in pots to study the influence of humic acid (humic acid (HA) and fulvic acid (FA)) on the accumulation of lead (Pb) and cadmium (Cd) in tobacco leaves. In non-polluted soils, the tobacco leaf uptake of Cd was dramatically influenced by the total soil metal concentration and soil properties, with no relationship observed for Pb. However, in soils that were polluted with 500 mg kg^{-1} of Pb or10 mg kg^{-1} of Cd, negative relationships were observed between the soil pH and the metal concentration in the tobacco leaves. When the soil was polluted with Pb, the application of 4 g kg^{-1} humic acids (HA or FA) decreased the leaf Pb concentration by 17.78%-48.32% in the acidic red and paddy soils but increased the concentration by 11.69%-37.54% in the alkaline cinnamon soil. A 8.74%-32.84% decrease in the leaf Cd concentration occurred in the acidic soils due to 4 g kg^{-1} HA, while there was a 4.45%-8.13% decrease and a 14.20%-46.37% increase in leaf Cd concentration in the yellow and cinnamon soils when HA and FA were applied, respectively. This finding suggests that the effects of humic acids on metal availability to plants in soil were pH-dependent, with inhibitory and stimulatory effects on acidic and alkaline soils, respectively. Therefore, humic acids (HA and FA) could be used to reduce Pb and Cd accumulation in plants growing in polluted acidic soil but not in alkaline soils.

Keywords: soil, tobacco leaf, Pb and Cd accumulation, humic acids

1. Introduction

With the development of industry, mining activity, and fertilizer application; the use of waste water for irrigation; and the application of sewage sludge, heavy metal soil contamination has become a worldwide concern. Lead (Pb) and cadmium (Cd) are non-essential elements and are potentially toxic to plants, animals, and humans. Researchers have devoted increasingly more resources to inhibiting the movement of Pb and Cd from the soil to the food chain (Keller *et al*., 2005; Yamato *et al*., 2008).

There are many methods available for reducing the accumulation of heavy metals in plants. Among these methods, an agricultural-based approach to in situ stabilization in which soil amendments are applied to remediate heavy metals, which is considered to be a relatively realistic and cost-effective choice (Lugon-Moulin *et al*., 2004). Humic substances are heterogeneous, high-molecular-weight organic substances that are composed of HA, FA, and humins, all of which have a different solubility in alkaline and acid solutions. Previous studies have indicated that both HA and FA have the capacity to bind metal ions and could thus be used for the in situ stabilization of metals (Benedetti *et al.*, 1996; Evangelou *et al*., 2004). The effects of humic acids on Pb and Cd accumulation in plants have been studied, but because of significant differences in soil types, plant species, and experimental formats, inconsistent (and sometimes contradictory) results have been reported(Yu *et al*., 2002; Evangelou *et al.*, 2004; Evangelou and Marsi, 2001; Janos *et al*., 2010; Jiang *et al.*, 2005; Topcuoglu, 2012 and Park *et al*., 2012). Thus, the effects of humic acids on the accumulation of Pb and Cd in plants require further investigation.

In the majority of previous studies, only one or two types of soil with similar properties were used (Kalis *et al.*, 2006; Li *et al*., 2003; Topcuoglu, 2012; Park *et al.*, 2012). Although it has been noted that the effects of humic acids on plant metal availability are pH-dependent (Evangelou and Marsi, 1999; Boruvka and Dradek, 2004), the scope and practice of humic acid application remain unexplored. This knowledge gap makes it difficult to correctly assess the efficacy of humic acid applications with regard to metal accumulation in plants.

In this study, potted tobacco experiments were conducted with four different soil types from China. The accumulation of Pb and Cd in the tobacco leaves from each soil was studied, the effects of humic acids on the accumulation of Pb and Cd in tobacco leaves grown in the different soils was explored, and the influence of humic acid application on plant metal availability was also observed.

2. Materials and Methods

2.1. Soil material

The four soils in this study are typical agricultural soil types in China. Topsoil samples of red, yellow, cinnamon, and paddy soil were collected from the Yunnan, Guizhou, Henan, and Fujian provinces, respectively. The soils were air-dried and passed through a 2-mm sieve prior to the experiments. The main chemical and physical properties of the soils,as well as their metal contents, were determined and are listed in Table 1.

2.2. Plant material

A flue-cured tobacco cultivar called Yunyan No.87 was used in this study. Tobacco seeds were surface-sterilized and germinated at 28°C. Germinated seeds were sown in vermiculite medium, and a floating system with a semi-Hoagland solution was employed to produce seedlings with seven or eight leaves each.

2.3. Humic acids

Humic acids (HAs) and fulvic acids (FAs) were purchased from the Tianjin Beifang Chemistry Company and the Henan Chemistry Institute, respectively.

2.4. Potted tobacco experiment

The following seven treatments were utilized for each soil type: Soil without any treatment (CK), 500 mg kg^{-1} Pb(Pb), 500 mg kg^{-1} Pb + 4g kg^{-1} HA(Pb+HA), 500 mg kg^{-1} Pb + 4 g kg^{-1} FA(Pb+FA), 10mg kg^{-1} Cd(Cd), 10 mg kg^{-1}Cd + 4 g kg^{-1} HA(Cd+HA), and 10 mg kg^{-1} Cd + 4 g kg^{-1} FA (Cd+FA). Each treatment was replicated three times, and the preparation procedure was as follows. First, 3 kg of soil that had been passed through a 2-mm sieve was placed in a 20 ×25-cm (Φ×H) plastic pot. Then, the Pb($Pb(NO_3)_2$), Cd($Cd(Cl_2)$), and pH-modified humic acids were added to the soil as solutions and maintained without additional water for 15d to reach equilibrium. After this equilibration period, seedlings with 7-8 leaves were transplanted to pots and grown at room

Table 1. Soil properties. Four types of soils with varying properties from China were used in this study. The topsoil samples of red, yellow, cinnamon, and paddy soils were collected from the Yunnan, Guizhou, Henan, and Fujian provinces, respectively. Different letters indicate a difference of $p = 0.05$.

Soil type	pH	Organic matter /(g kg^{-1})	$CaCO_3$ /(g kg^{-1})	Clay content /(g kg^{-1})	Total Pb /(mg kg^{-1})	Total Cd /(mg kg^{-1})
Paddy soil	5.18c	24.75c	0.63c	260.51c	40.76a	0.26b
Red soil	5.31c	32.87b	2.44b	576.43b	27.61c	0.25b
Yellow soil	6.06b	37.56a	2.48b	613.44a	35.76b	1.45a
Cinnamon soil	7.91a	11.42d	22.71a	273.75c	17.01d	0.22b

temperature for 45d. At the end of the experiment, the plants were carefully harvested and rinsed with water and de-ionized water. The leaves, shoots, and roots were separated and dried at 80°C for 48 h, and the samples were subsequently analyzed for Cd and Pb.

2.5. Pb and Cd determination

Total tobacco leaf metal concentrations were analyzed by AAS according to the methods recommended by Perkin Elmer. Dried plant samples (0.2g) were digested with HNO_3-H_2O_2 (5:2 v/v) by microwave digestion (Milestone ETHOS) and diluted with de-ionized water to 50 ml. The Pb and Cd concentrations were measured by GF-AAS (PE-800).

2.6. Statistical analysis

Metal concentrations were obtained in triplicate for each treatment. The differences in average concentrations for each treatment were statistically evaluated by ANOVA (p=0.05) using Duncan's multiple range tests (SPSS V.13.0).

3. Results

3.1. Pb and Cd accumulation in tobacco leaves from different soils

3.1.1. Pb accumulation

The Pb concentrations of tobacco leaves grown in the four different soils under non-polluted conditions are shown in Figure 1A. Although the total soil Pb concentration ranged from 17.01 to 40.76 mg·kg^{-1}, no significant difference was observed in the Pb concentration of tobacco leaves across the four distinct soils. Furthermore, no relationship was observed between the leaf Pb concentrations and the soil properties. This finding indicated that the influence of soil properties on tobacco leaf Pb accumulation was limited in soils without obvious contamination.

When the soil was polluted with 500 mg kg^{-1} Pb, the Pb concentration of the tobacco leaves was 4.45 (red soil), 4.45 (paddy soil), 1.71 (yellow soil), and 1.47 (cinnamon soil) times greater than that of the controls grown in unpolluted soil. Meanwhile, the Pb concentration in the leaves that were grown in acid red and paddy soil was significantly higher than that in the leaves grown in yellow and cinnamon soils (Figure1B).

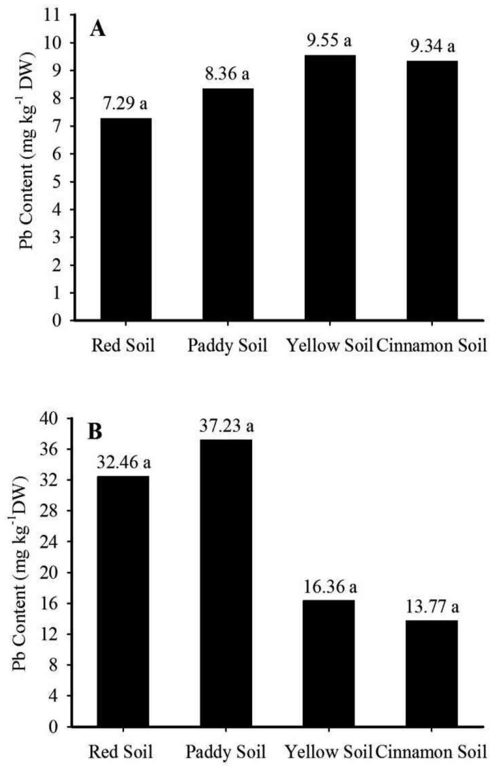

Figure 1. A) Pb concentration of tobacco leaves grown in non-polluted soils; B) Pb concentration of leaves grown in polluted (500 mg kg^{-1} Pb) soils. Tobacco seedlings with 7-8 leaves were grown for 45 d in pots with non-polluted or polluted (500 mg kg^{-1} Pb) red, paddy, yellow, or cinnamon soil. The Pb contents of the tobacco leaves were determined. Data are presented here as the means of three replicates. Different letters represent a difference with p=0.05.

3.1.2 Cd accumulation

Unlike Pb, the total soil Cd levels positively influenced cadmium accumulation in tobacco leaves under non-polluted conditions (Figure 2A). For plants grown in the yellow soil, which had a higher total soil Cd concentration, the leaf Cd concentrations were 1.53-, 1,65-, and 5,19-fold greater than those in plants grown in the red, paddy, and cinnamon soils, respectively. This result indicates that soil Cd was a key factor in tobacco leaf Cd accumulation. In addition, among the three types of soils with similar total Cd levels, the Cd concentration in leaves from plants grown in the cinnamon soil, which had the highest pH and calcium carbonate content, was the lowest (approximately1/3 that in leaves grown in red and paddy soils), suggesting that soil pH and calcium carbonate content also played important roles in tobacco leaf Cd accumulation.

When soils were polluted with 10 mg kg^{-1} Cd, the leaf Cd concentration was increased to 57.93-118.78 mg kg^{-1} and was 24.0 (red soil), 26.0 (paddy soil), 10.0 (yellow soil), and 40.2 (cinnamon soil) times greater than that of the non-polluted controls. And as with the Pb results, the leaf Cd concentrations were significantly higher in the acidic red and paddy soils than in the yellow and cinnamon soils with a higher pH (Figure 2B).

3.1.3 Relationship between tobacco leaf Pb and Cd concentration and soil pH

As shown in Figure 3, negative correlations were observed both between the leaf Pb concentration and the soil pH ($r^2 = 0.715$) (Figure 3A) and between the soil pH and leaf Cd concentration ($r^2 = 0.832$) under polluted conditions (Figure 3 B). These results indicated that soil pH plays a key role in the accumulation of Pb and Cd in tobacco leaves grown in contaminated soils.

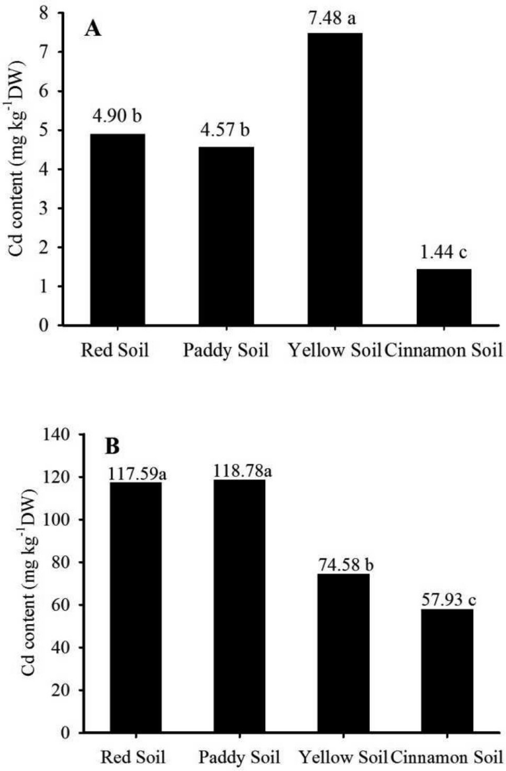

Figure 2. A) Cd concentration of tobacco leaves grown in non-polluted soils; B) Cd concentration of tobacco leaves grown in polluted(10 mg kg^{-1} Cd) soils. Tobacco seedlings with 7-8 leaves were grown for 45d in pots with non-polluted or polluted (10 mg kg^{-1} Cd) red, paddy, yellow, or cinnamon soil. The Cd contents of the tobacco leaves were determined. Data are presented as the means of three replicates. Different letters represent a difference with a significance of $p = 0.05$.

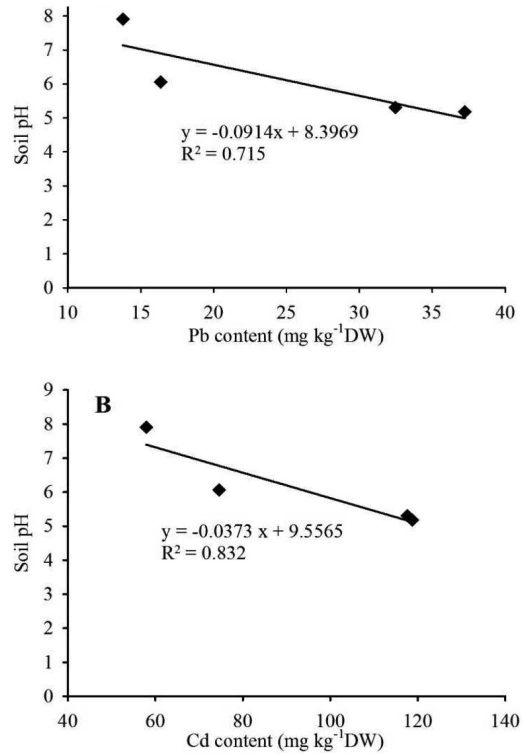

Figure 3. A) The relationship between soil pH with tobacco leaf Pb concentration and B) Cd concentration in polluted soils. Tobacco seedlings with 7-8 leaves were grown for 45d in soils polluted with 500 mg kg^{-1} Pb or 10 mg kg^{-1} Cd in pots containing red, paddy, yellow, or cinnamon soil.

3.2. The effects of humic acids on Pb and Cd accumulation in tobacco leaves

3.2.1. Plant biomass

It is generally accepted that trace amounts of heavy metals stimulate plant growth, while high concentrations of metals can cause plant injury.

In our experiments, 500 mg kg^{-1} Pb caused injurious effects on tobacco growth, and the plant biomass of the red, paddy, yellow, and cinnamon soils decreased by 20.25%, 20.43%, 13.92%, and 20.18%,respectively,compared with that of the unpolluted controls (Figure 4A). No significant differences were observed between treatments with or without humic acids. When 10mg kg^{-1} Cd was added, the tobacco plant biomass decreased slightly, and no significant difference was observed between almost all of the treatments (Figure 4B), which indicated that 10 mg kg^{-1} Cd had no significant influence on tobacco growth.

3.2.2. Effects of humic acids on tobacco leaf Pb accumulation

The Pb concentration of leaves grown in Pb-polluted soils with and without the addition of humic acids is shown in Figure 5A. Compared with the results derived from treatment without humic acids, the leaf Pb concentration decreased by 19.44% and 30.22% for HA and 17.78% and 48.32% for FA in leaves grown in the acidic red and paddy soils, respectively. These results suggest that humic acids can effectively reduce Pb uptake by tobacco in polluted acid soils. However, in the alkaline cinnamon soil, the addition of HA and FA increased the leaf Pb concentration by 11.69% and 37.54%, respectively, which indicated a stimulatory effect. Furthermore, the leaf Pb concentration increased slightly in the presence of HA but decreased by 62.65% in the presence of FA in the yellow soil. Thus, the effects of humic acids on Pb accumulation in tobacco grown in yellow soil should be further investigated.

3.2.3. Effects of humic acids on tobacco leaf Cd accumulation

In soils polluted with 10 mg·kg^{-1} Cd, the addition of 4g kg^{-1} HA decreased the tobacco leaf Cd concentration by 8.74%,11.03%,4.45%, and 8.13%

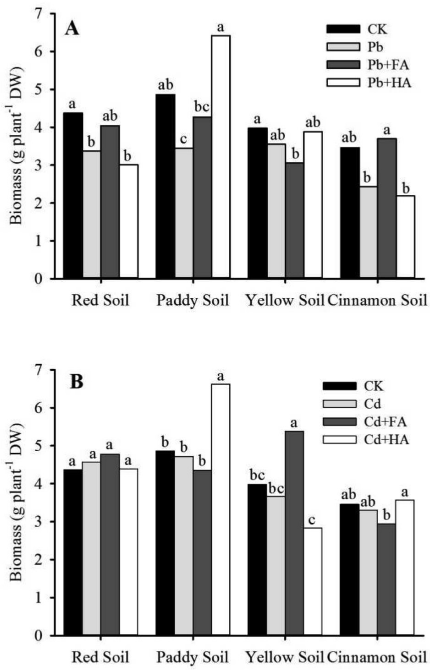

Figure 4. A) The tobacco biomass of Pb and B) Cd treated soils. Tobacco seedlings with 7-8 leaves were grown for 45d in red, paddy, yellow,or cinnamon soil with either the CK, Pb, Pb+FA, Pb+HA, Cd, Cd+FA, or Cd+HA treatment, representing treatments of 500 mg kg^{-1} Pb, 500 mg kg^{-1} Pb + 4 g kg^{-1} FA, 500 mg kg^{-1} Pb + 4 g kg^{-1} HA, 10 mg kg^{-1} Cd, 10 mg kg^{-1} Cd +4 g kg^{-1} FA, and 10 mg kg^{-1} Cd + 4 g kg^{-1} HA, respectively The biomass of the tobacco leaves was determined. Data are presented as the means of three replicates. Different letters represent a difference of p = 0.05.

in plants grown in red, paddy, yellow, and cinnamon soils, respectively (Figure 5B), demonstrating an inhibitory effect on Cd accumulation in tobacco leaves.

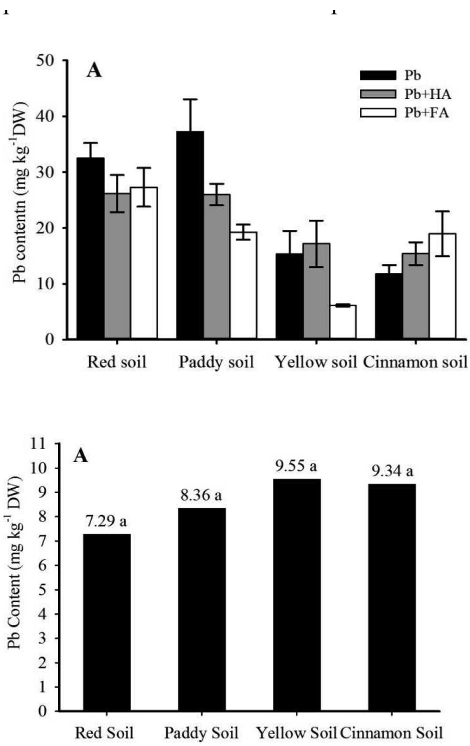

Figure 5. A) The effects of humic acids on tobacco leaf Pb accumulation; B) The effects of humic acids on tobacco leaf Cd accumulation. Tobacco seedlings with 7-8 leaves were grown for 45d in red, paddy, yellow, or cinnamon soil with either the Pb, Pb+FA, Pb+HA, Cd, Cd+FA, or Cd+HA treatment, representing the 500 mg kg^{-1} Pb, 500 mg kg^{-1} Pb + 4 g kg^{-1} FA, 500 mg kg^{-1} Pb + 4 g kg^{-1} HA, 10 mg kg^{-1} Cd, 10 mg kg^{-1} Cd +4 g kg^{-1} FA, and 10 mg kg^{-1} Cd + 4 g kg^{-1} HA treatments ,respectively. The Cd contents of tobacco leaves were determined. Data are presented as the means of three replicates. Data are presented as the means ±SE (n=3).

When FA was applied, the tobacco leaf Cd concentration decreased by 13.58% and 32.84% in the acidic red and paddy soils, respectively, while it increased by 46.37% and 14.20% in the yellow and cinnamon soils (with higher pH values), respectively. This finding suggests that the effects of humic acids on Cd accumulation in tobacco leaves were pH-dependent, and humic acids exhibited inhibitory and stimulatory effects on Cd accumulation in tobacco leaves grown in acidic and alkaline soils, respectively.

4. Discussion

4.1. Cd and Pb accumulation in plants with respect to soil properties

Plant Cd and Pb accumulation is influenced by many factors, such as soil pH, organic matter content, and soil metal concentration.

Our study first addressed the Pb and Cd accumulation in tobacco leaves grown under non-polluted conditions in four types of typical Chinese agricultural soils with varying properties. The results demonstrated very different phenomena with respect to the Pb and Cd accumulation in tobacco leaves. The tobacco leaves grown in yellow soil with a higher Cd concentration exhibited significantly higher Cd concentrations. In soils with similar Cd levels, the pH played a vital role in metal uptake. This finding is similar to results in the literature (Strobel *et al*., 2005; Meers *et al*., 2005; McBride, 2002; Gray *et al*., 1999) showing that Cd availability to plants in soils is influenced by soil properties. Recent studies even proposed that Cd uptake by crops could be estimated from the total soil Cd, soil pH, and % humus (McBride, 2002; Hough *et al*., 2003). Thus, soil property modification was an effective way to reduce Cd accumulation in plants under non-polluted conditions. However, Pb accumulation in tobacco had no relationship with soil properties in our experiment, as was the case in winter wheat (Hough *et al.*, 2003), but not soil solution (Yang *et al*., 2010; Reddy *et al.*, 1995), results. It was reported that Pb had lower soil availability (Yang *et al*., 2010) and that aerial deposition was the major reason for Cd accumulation in plants, especially in urban areas (Zhao *et al*., 2012). As a consequence, the effects of soil properties on Pb accumulation in plants maybe concealed by other factors, resulting in inconsistent results between the soil solution and plant accumulation data. Thus, soil property modification appeared to not be as effective for plants for controlling Pb uptake from non-polluted soils.

Nevertheless, the soil pH had a vital influence on the accumulation of Pb and Cd under contaminated conditions in tobacco leaves, and the accumulation of Cd and Pb in tobacco leaves decreased with increasing pH, which is consistent with the literature (Kamewada and Nakayama, 2009; Hough *et al*., 2003; Dayton *et al*., 2006). This result indicated that Pb and Cd pollution in acidic soils yielded a higher environmental risk and suggested that efforts to increase soil pH will effectively decrease both Cd and Pb accumulation in plants grown in polluted acidic soils.

It has been reported that the availability of metal is higher in polluted soils than in clean soils (Yang *et al*., 2010, Janos *et al*., 2010), as well as that the availability of Pb is notably lower than that of Cd (Andersen *et al*., 2002). In our previous studies with the same four types of soil, the extractable Pb and Cd content was less than 1% and more than 20% of the total metal content in the non-polluted soils, respectively, and more than 5% and 40% of the total metal content in the contaminated soils, respectively (Yang *et al*., 2010). This finding indicates that there is a close relationship between soil properties, especially soil pH, and metal accumulation in plants. This finding is especially true of metals with higher availability in soil under polluted conditions or of metals that are weakly bound to the soil. Under these conditions, the practice of soil property modifications, especially pH modification, was an effective way to reduce metal accumulation in plants. However, under the non-polluted conditions in which the metal was strongly bound to soil particles, soil property modification appeared to be less useful.

4.2. Humic acid effects on Pb and Cd accumulation in plants

The effects of humic acids on metal speciation in soil and then the availability to plants have been widely explored. However, because of the complex structure of humic acids and significant differences in soil properties, inconsistent or even contradictory results have been reported (Evangelou and Marsi, 2001; Yu *et al.*, 2002; Janos *et al*., 2010; Jiang *et al*., 2005; Evangelou *et al*., 2004; Topcuoglu, 2012; Park *et al.*, 2012; Li *et al.*, 2003; Gao *et al*., 2009). Some experiments have shown that the addition of humic acids lowered the free and labile metal concentrations of the soil (Yu *et al.*, 2002; Janos *et al*., 2010; Jiang *et al*., 2005, Li *et al*., 2003; Gao *et al*., 2009) and inhibited plant Pb and Cd accumulation (Kalis *et al*., 2006; Li *et al*., 2003), suggesting that humic acids could be used to immobilize Pb and Cd in the soil. Nevertheless, the results of other experiments have demonstrated the stimulatory effects of humic acids on the accumulation of Cd and Pb in plants (Evangelou *et al*., 2004; Topcuoglu, 2012; Park *et al*., 2012) and suggested that humic acids are effective substances for metal bioremediation/extraction from soil. This makes it difficult to evaluate the efficacy of humic acid application on metal immobilization or bioremediation.

Both inhibitory and stimulatory effects of humic acids on Cd and Pb accumulation were observed in tobacco leaves in the four types of soils with varying soil properties. The application of HA and FA effectively reduced Pb and Cd accumulation in tobacco leaves grown in the acid red and paddy soils. A significant stimulatory effect of alkaline and calcareous cinnamon soil was observed, with more complicated phenomena observed for the yellow soil, which had a higher organic content and a neutral pH. This finding was consistent with the results of Clemente and Bernal (2006), who reported that humic acids caused significant Pb immobilization in acidic soil, with lesser effects in calcareous soil. This consistency indicates that the effects of humic acids on metal availability and immobilization/bioremediation should be investigated in more detail.

It has been noted that humic acids have multiple functional groups that can interact with metal ions to form metal-humic complexes, and their effects on metal mobility in soil and their availability to plants depend on the nature/strength of the metal complex (Datta *et al*., 2001; Evangelou and Marsi, 2001). The strength of metal-ion humic complexes was highly pH-dependent, and humic acids generally increased metal adsorption at low pH levels (Evangelou and Marsi, 1999; Boruvka and Dradek, 2004; Waller and Pickering, 1993). In the literature, the inhibitory effects of humic acids on metal availability were always reported in acidic soils (Yu *et al*., 2002; Jiang *et al*., 2005; Li *et al*., 2003; Gao *et al*., 2009), while most of the stimulatory effects of humic acids on metal availability were observed in alkaline soils (Evangelou *et al*., 2004; Topcuoglu, 2012; Park *et al*., 2012). This trend may reflect the existence of different mechanisms of humic acid action on metal availability under acidic and alkaline conditions. Low pH increased the metal sorption of humic acids (Boruvka and Drabek, 2004).The adsorption action between metal and humic acids were predominant under acidic conditions, and, as a result, the inhibitory effects on metal availability were always observed. However, the decrease in the soil pH due to the addition of humic acids in alkaline soil may be important (Evangelou *et al*., 2004), and a stimulatory effect on metal availability was observed. Thus, we concluded that in the polluted acidic soils, humic acids mainly inhibited plant metal availability and could be used to reduce plant metal accumulation. In alkaline soils, the humic acid treatments exerted a stimulatory effect on plant metal availability and were well suited for metal bioremediation.

5. Conclusions

On the conditions of metals with higher availability in the soil which related to polluted conditions or metals that were weakly bound to soil, the practices of soil property modification, especially pH modification, was an effective way to reduce metal accumulation in plants. The effects of humic acids on Pb and Cd were pH-dependent. The application of HA and FA effectively reduced Pb and Cd accumulation in tobacco leaves from the acidic red and paddy soils, while a major stimulatory effect was observed on leaves grown in the alkaline and calcareous cinnamon soil. As a result, humic acids could be used to reduce metal accumulation in plants growing in polluted acidic soils and could be used for metal bioremediation in alkaline soils.

Acknowledgments

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