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Full Length Research Paper

Cu and Pb accumulation in maize (*Zea mays* L.) and soybean (*Glycine max* L.) as affected by N, P and K application

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Nitrogen (N), phosphorus (P) and potassium (K) fertilizer application affects soil properties and processes in many ways that remain unclear. The effects of N, P, and K application on plant growth and copper (Cu) and lead (Pb) accumulation, as well as their differences in plant species were thus evaluated in soil- maize and soil-soybean systems. Five treatments were arranged: N, P, and K application (NPK), N and P application (NP), N and K application (NK), P and K application (PK), and CK (no fertilization). Results showed that N application could significantly increase maize shoot Cu concentration and translocation from soil to above ground tissue ($p<0.05$). However, soybean shoot Cu concentration could not increase with N application. Phosphorus application could effectively decrease the bioavailability of Cu and Pb in both systems. Under nutrients deficiency, soil dissolved organic carbon (C) significantly increased, showing difference in plant species ($p<0.05$), which further enhanced Cu and Pb mobility in soils. Copper removal from soil positively correlated with plant biomass production with the NPK and NP treatments showing the greatest in both crops. Our results show that, an appropriate increase in P application and decrease in N application are recommended to reduce agro-ecological risks associated with Cu and Pb in soil-plant systems.

Key words: Soil-plant system, heavy metals, Cu and Pb mobility, ecological risk, translocation.

INTRODUCTION

Pollution induced by heavy metals in soils is a serious environmental problem and has received increasing attention in recent years due to their toxicity to both the ecosystem and human health (Ahmed and Bouhadjera, 2010). At present, remediation technologies for soils contaminated by heavy metals are of particular interest, and effective management demands an understanding of various processes within soil-plant systems (Wei et al., 2008; Kim et al., 2010a). The transformation of heavy Cu (Pb) concentration to root Cu (Pb) concentration metals is influenced by soil composition, physicochemical properties and plant growth, as well as soil-plant interfacial interactions (Quartacci et al., 2009; Wu et al., 2010).

N, P and K are among the most important nutrients for plant growth, and their diverse concentrations have a significant influence on the properties of soil-plant interface. Nutrient deficiency in soil can stimulate the release of exudates and alter plant growth patterns (Chaignon et al., 2002; Morgan et al., 2005). Recent studies have proved that, dissolved soil organic matter have the significant effects on transformation of heavy metals through the increment of heavy metal solubility, root growth and plant uptake (Quartacci et al., 2009; Kim et al., 2010b). Plant transpiration, photosynthesis, amino acid metabolism and biomass production, which all directly or indirectly regulate the behaviors of heavy

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Abbreviations: DTPA, Diethylene trinitrilopentaacetic acid; **DOC,** soil dissolved organic C; **S/R of Cu (Pb),** the ratio of shoot.

metals in soil- plant system (Jarvis and Whitehead, 1981; Salah and Barrington, 2006; Ruley et al., 2006), are also affected by N, P and K application. On the other hand, the bioavailability of heavy metals varied in soils with different fertilization management owing to the differences in soil pH, organic matter content, microbial activity and heavy metal mobilization (Ma et al., 1993; Singh et al., 2010; Wu et al., 2010). Phosphorus application was successfully used to reduce environmental risks in Pb-polluted areas (Ma et al., 1993; Ma and Rao, 1997). Thus, the optimization of nutrient management is of great significance for reducing agro-ecological risks associated with heavy metals pollution (Tu et al., 2000).

To date however, the effect of nutrient supply on the behaviors of heavy metals in soil-plant system, such as heavy metal availability, plant uptake, and translocation, is still not fully understood. The response of plant growth to N, P and K application varies in species, which in turn suggests the idea that the influences of N, P and K on the behaviors of heavy metals in soil-plant system differed in plant species. In the present study, the effects of N, P and K on the transformation of Cu and Pb in two soil-plant systems, namely, soybean (*Glycine max* L.) and maize (*Zea mays* L.) was thus investigated, under different N, P, and K application schemes. The results in terms of soil-plant properties and metal accumulation, as well as their differences between two species were discussed.

MATERIALS AND METHODS

Soil

The soil, classified as Aquic Inceptisol, was collected from topsoil (0~20 cm) in an agricultural field, which had a sandy loam texture (about 9% clay, 22.8% silt). It was air-dried, ground and sieved (<2 mm). In the pretreated soil, organic C, total N and P contents were 8.82, 0.45 and 0.45 g kg^{-1}, respectively. Soil available N, P and K contents were 10.85, 6.50 and 46.58 mg kg^{-1}, and pH was 7.50. Total Cu and Pb concentrations were 14.5 and 21.9 mg kg^{-1}, respectively. Chemical analysis methods used to acquire pretreated soil properties are given shortly.

Experimental design

The soil used in this study was amended with Cu^{2+} (100 mg kg^{-1}, $CuCl_2 \cdot 2H_2O$) and Pb^{2+} (100 mg kg^{-1}, $PbCl_2$) dissolved in water. The contaminated soil was shaken and sieved through a 2-mm sieve again to distribute Cu^{2+} and Pb^{2+} homogeneously, and then were stored for 30 days under alternating wet and dry conditions (Blaylock et al., 1997) . In order to determine soil-plant interactions and transformation of heavy metals in the rhizosphere, a rhizobag was used. A root bag (4.1 cm diameter and 14 cm in height) made of 500- mesh nylon cloth was filled with 0.23 kg of polluted soil and placed along the central geometric axis inside a plastic pot (14 cm diameter and 14 cm in height). After rinsing with deionized water, the germinated seeds of Taiyu-2 maize (*Z. mays* L.) cultivar and Ludou-11 soybean (*G. max* L.) cultivar were sown in the pots. The seedlings were subsequently thinned to 1 plant per pot after emergence and grown under greenhouse conditions ranged from 20 to 30°C during the day and 15 to 20°C at night. Inorganic salt solution was used weekly as nutrient sources for plants, which involved five treatments in triplicate, that is, NPK (80 mg N kg^{-1}, 35 mg P kg^{-1} and 60 mg K kg^{-1}), NP (80 mg N kg^{-1} and 35 mg P kg^{-1}), NK (80 mg N kg^{-1} and 60 mg K kg^{-1}), PK (35 mg P kg^{-1} and 60 mg K kg^{-1}) and CK (deionized water only).

To avoid water leaking from pots, 300 ml of inorganic salt solutions were applied when watering, which adjusted soil moisture percent to approximately 60% of the water holding capacity. Deionized water was used between nutrient applications, if plants were experiencing water stress. Inorganic N, P and K nutrients were applied as urea, NaH_2PO_4 and KCl, respectively. At day 45 after sowing, plant roots and shoots were harvested and separated from the soil, washed with running water, rinsed thoroughly with deionized water, and then dried at 60°C for 48 h. The dry biomasses were measured before heavy metal determination. Topsoils (0~2 cm) in the root bags which were not in close proximity to roots were discarded. The residuals were collected and stored at 4°C until analysis.

Heavy metal analysis

Because drying affects heavy metal forms in soils (Bordas and Bourg, 1998), the diethylene trinitrilopentaacetic acid (DTPA) extraction procedure was used to determine Cu and Pb concentrations for fresh soils (Su et al., 2004). Shoot or root samples were digested with HNO_3-$HClO_4$-H_2SO_4 (7:2:1, v/v) to determine the total heavy metal concentrations (Zhou et al., 2007). The concentrations of Cu and Pb in digested solutions and DTPA extraction were measured by an atomic absorption spectrophotometer equipped with a graphite furnace (Shimadzu, AAS 6800).

Soil analysis

Soil pH was measured in a soil-water suspension (1:2.5 v/v). Available N was extracted with 2 mol L^{-1} KCl and analyzed with a Segmented Flow Analyzer (AAA, German). Available P and available K were extracted by sodium bicarbonate and ammonium acetate, respectively (Lu, 2000). Soil dissolved organic C (DOC) content was extracted with ultrapure water and measured by a liquid TOC II analyzer (Elementar, Germany) after filtration through a 0.45 m millipore filter (Martín-Olmedo and Rees, 1999).

Statistical analysis

Shoot: root ratio (S/R) of Cu or Pb was calculated as the ratio of the shoot Cu or Pb concentration to the root Cu or Pb concentration. Total Cu or Pb accumulation in maize or soybean was the sum of metals in shoot and root. All data are mean values from three replicates. Using SPSS software (12.0), differences between means were determined by LSD test at $p<0.05$.

RESULTS

Soil properties

The lowest soil pH both in soil-maize and soil-soybean systems was observed in the NK treatments, which was significantly lower than other four treatments, followed by

Table 1. Soil parameters showing the effect of various fertilizer treatments in a controlled greenhouse experiment.

Fertilizer application treatments [a]	pH (H_2O)		DOC [b] (mg kg^{-1})		Available N (mg kg^{-1})		Available P (mg kg^{-1})		Available K (mg kg^{-1})	
	Maize [c]	Soybean [d]	Maize	Soybean	Maize	Soybean	Maize	Soybean	Maize	Soybean
NPK	7.24 b	7.25 b	17.05 b	19.61 b	64.15 c	70.63 b	23.98 b	18.93 b	124.44 a	119.32 a
NP	7.31 b	7.26 b	16.02 bc	18.06 b	83.45 b	88.78 b	28.92 b	21.60 a	54.72 d	45.26 c
NK	7.15 c	7.16 c	17.27 b	23.32 a	114.17 a	106.37 a	9.42 c	10.12 c	92.53 b	127.83 a
PK	7.50 a	7.43 a	15.06 c	15.88 c	5.40 d	8.55 c	35.30 a	22.43 a	66.64 c	73.91 b
CK	7.44 a	7.39 ab	18.77 a	17.54 bc	5.65 d	7.51 c	7.71 c	7.94 c	20.59 e	39.25 c

[a] PK, N, P, and K application; NP, N and P application; NK, N and K application; PK, P and K application; CK, no fertilization. [b] DOC, soil dissolved organic C. [c] Maize, soil-maize system. [d] Soybean, soil-soybean system. Means (n = 3) with the same letter within columns are not significantly differed at $p < 0.05$.

the NPK and NP treatments, and the greatest in the PK treatment (Table 1). The greatest DOC content was in the NK treatment in soil-soybean system, while in soil-maize system, the greatest was in the CK treatment. Available soil N, P or K contents were higher in the treatments where N, P or K was applied.

Plant biomass production

Maize growth was significantly influenced by various fertilizer applications (Figure 1a). The highest total maize weight was in the NPK and NP treatments, followed by the PK treatment, and the least in the NK and CK treatments. Compared to the CK treatment, the dry weight of maize shoot was significantly greater in the fertilized treatments. For maize root, however, the greatest to least dry weights were NPK>PK>NP>CK>NK.

In soil-soybean system, the dry shoot, root and total weight were relatively little affected by fertilizer application in comparison to maize (Figure 1b). No significant difference in dry weight was observed, except that shoot dry weight in the NK treatment was significantly lower than the other four treatments.

Cu and Pb accumulation in plants

The maize shoot Cu concentrations in the N-fertilized treatments were significantly higher than those in the treatments without N application, which was about twice as high as in the PK treatment (Table 2) . The greatest maize shoot Cu concentration was found in the NP treatment, followed by the NPK and NK treatments, the least in the PK treatment. However, in soil- soybean system, the shoot Cu concentration in the NK treatment was the highest, which was significantly higher than that in the NPK, NP and PK treatments (Table 2). Cu concentrations in maize and soybean roots were far higher than those in plant shoots, and the greatest was both in the NK treatment.

In maize-soil system, S/R of Cu was significantly higher in the N -fertilized treatments (NPK, NP and NK), and the least was in the PK treatment. Total maize Cu accumulation in the NPK and NP treatments was significantly higher than that in other three treatments, with the least in the NK treatment. For soybean-soil system, S/R of Cu varied significantly among treatments. The greatest was in the NK treatment, followed by the CK treatment, and the least in the NPK and NK treatments. The greatest total soybean Cu accumulation was in the NPK treatment, which was significantly higher than that in the PK and CK treatments (Table 2). The shoot or root Pb concentrations in the treatments without P application were significantly higher than those in the P-fertilized treatments, except for maize root Pb concentration in the NPK treatment (Table 2). The greatest maize shoot Pb concentration was in the CK treatment, while the greatest soybean shoot Pb concentration was in the NK treatment. They were both significantly higher than that in other four treatments. Lead concentrations in maize and soybean roots were far higher than those in the shoot, which ranged from 57.7 to 79.4 mg kg^{-1} in maize and from 64.1 to 108.6 mg kg^{-1} in soybean. The greatest maize and soybean root Pb contents were both in the NK treatment.

Maize or soybean S/R of Pb in the P-fertilized treatments was significantly lower than that in the treatments without P application. The least total maize Pb accumulation was in the NK treatment, which was significantly lower than that in the NPK treatment. The greater total soybean Pb accumulation was in the treatments without P application, which was significantly higher than that in the NP and PK treatments (Table 2).

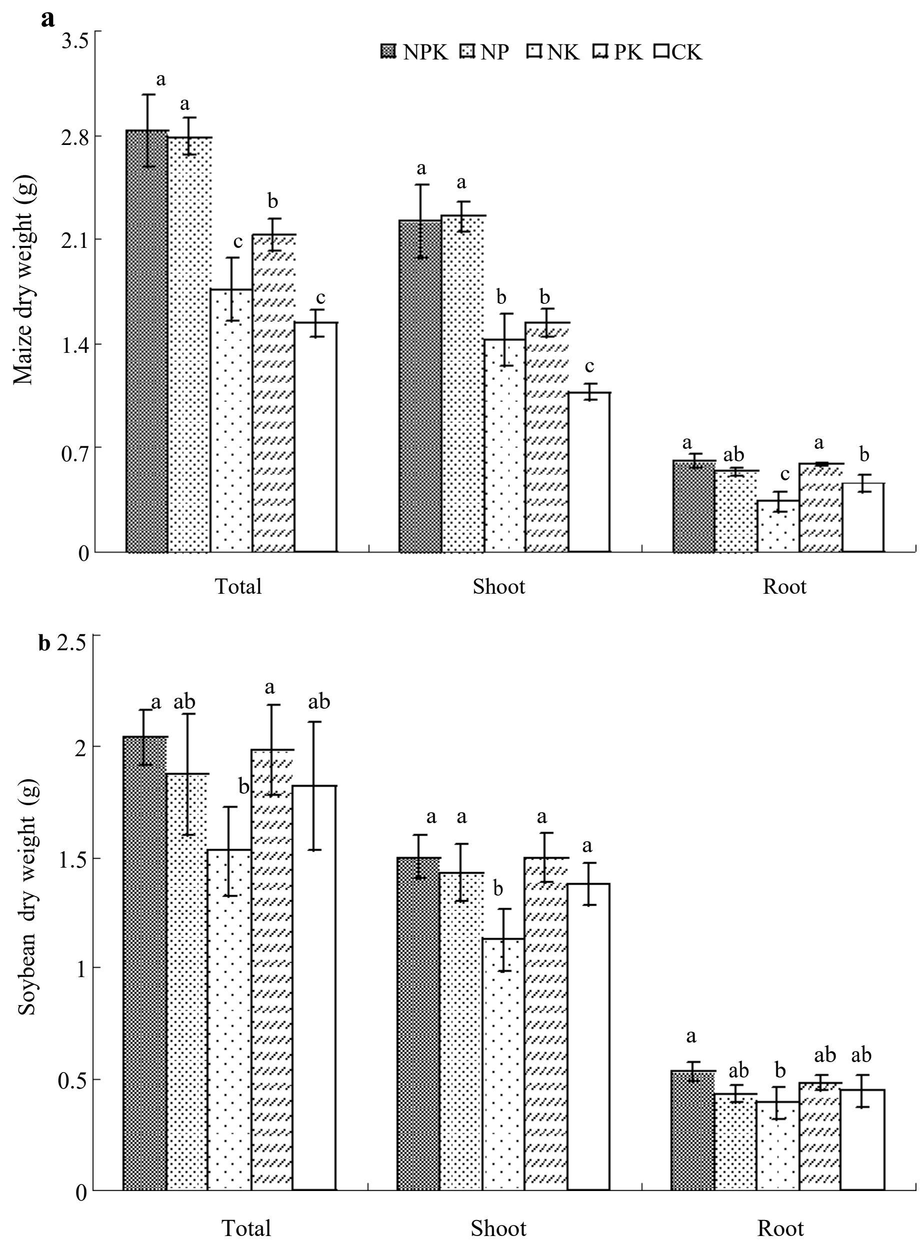

Figure 1. Maize and soybean dry biomass showing the effect of various fertilizer treatments in a controlled greenhouse experiment: (a) Maize (b) Soybean. NPK, N, P, and K application; NP, N and P application; NK, N and K application; PK, P and K application; CK, no fertilization. Total, the whole plant weight; Shoot, shoot weight; Root, root weight. Columns represent means (n = 3) and bars on each column represent standard deviation; means with the same letter within groups are not different at $p < 0.05$.

DTPA extractable metals in soils

Soil Cu and Pb concentrations extracted by DTPA are shown in Figure 2. The greatest DTPA extractable Cu and Pb were both in the NK treatment. The least DTPA extractable Cu was in the PK treatment, showing significantly lower values than the other four treatments in maize-soil system (Figure 2a). DTPA extractable Pb was significantly greater in the treatments without P application than that in the P-fertilized soils, and no significant difference was observed among P-fertilized treatments (Figure 2b) .

DISCUSSION

Effect of NPK on soil properties and plant growth

It is no doubt that soil N, P, and K concentrations

Table 2. Cu and Pb accumulation in plants showing the effect of various fertilizer treatments in a controlled greenhouse experiment.

Fertilizer application treatments [a]	Cu				Pb			
	Shoot [b] (mg kg^{-1})	Root [c] (mg kg^{-1})	Total [d] (g)	S/R [e]	Shoot (mg kg^{-1})	Root (mg kg^{-1})	Total (g)	S/R
Maize								
NPK	15.10 ab	277.2 a	201.7 a	0.055 ab	5.30 c	63.0 ab	50.3 a	0.084 c
NP	17.80 a	279.4 a	190.8 a	0.064 a	4.84 c	57.7 b	41.9 ab	0.084 c
NK	14.99 b	302.8 a	121.8 c	0.050 bc	8.75 b	79.4 a	40.4 b	0.110 b
PK	7.73 d	248.4 a	158.5 b	0.031 d	5.12 c	59.9 ab	43.1 ab	0.085 c
CK	10.61 c	243.7 a	122.4 c	0.044 c	10.16 a	74.7 a	45.1 ab	0.135 a
Soybean								
NPK	8.78 b	150.1 b	93.5 a	0.058 cd	4.40 c	70.3 c	43.9 ab	0.062 d
NP	8.78 b	159.6 ab	83.1 ab	0.055 d	4.52 c	66.9 c	35.5 b	0.067 d
NK	13.07 a	169.8 a	81.8 b	0.077 a	11.3 a	98.6 a	51.8 a	0.115 a
PK	8.57 b	124.0 c	73.2 bc	0.069 bc	5.20 c	64.1 c	38.4 b	0.088 c
CK	9.96 ab	135.3 bc	73.9 bc	0.074 ab	9.21 b	91.9 ab	53.5 a	0.100 b

[a] NPK, N, P, and K application; NP, N and P application; NK, N and K application; PK, P and K application; CK, no fertilization.[b] Shoot, Cu or Pb concentration in plant shoots. [c] Root, Cu or Pb concentration in plant roots. [d] Total, Cu or Pb accumulation in whole plant. [e] S/R, the ratio of the shoot Cu or Pb concentration to the root Cu or Pb concentration. Means (n = 3) with the same letter within columns in each group are not significantly differed at $p < 0.05$.

increase with N, P, and K application. DOC was mainly from root decomposition products and exudates in soil (Jones, 1998). As a result of low root dry weight, the greatest DOC in maize CK and soybean NK treatments was probably due to the enhancement of root exudates under nutrient deficiency (Zhang et al., 1997). The difference of DOC between both systems suggested that, the response of plants to nutrient deficiency varied in species (Dechassa and Schenk, 2004). Unbalanced N fertilization can lead to soil pH decrease (Guo et al., 2010), with the NK treatment showing the greatest N accumulation and the lowest pH. Meanwhile, more OH^- can be released from soil colloids in the presence of phosphate ions. Hence, soil pH increased to the greatest in the PK treatment which had the highest available P concentration.

Fertilization, especially N fertilization, significantly increased the total maize biomass production, but root growth was significantly inhibited in the NK treatment owing to soil available N accumulation (Li et al., 2009). Compared to maize, soybean dry weight of the total, shoot and root received less decrement in the treatments without N application. Because legume crops, such as soybean, can increase soil N content through N_2 fixation which can be as N source to facilitate plant growth, especially in N-poor soils (Rodríguez et al., 2009).

Effect of NPK on Cu and Pb accumulation in plants

The transformation of heavy metals in soil-plant system was relative to nutrient treatments (Tu et al., 2000; Lin et al., 2010). Bioavailability of heavy metals in soil is positively correlated with their DTPA extractable quantity (Conesa et al., 2007). In the present study, the greatest soil DTPA-extractable Cu and Pb concentrations were both in the NK treatment. These might be caused by the soil DOC increase and pH decrease under N-unbalanced fertilization condition. Wong et al. (2007) found that, DOC could significantly reduce metal sorption and increase its mobility through the formation of soluble DOC-metal complexes in soils. Soil acidification also can improve heavy metal mobility (Pérez-Novo et al., 2009). The relationship analysis indicated that, DOC positively correlated with DTPA-extractable Cu and Pb, which reached the significant level in soil-maize system and was in close proximity to the significant level in soil-soybean system (Tables 3

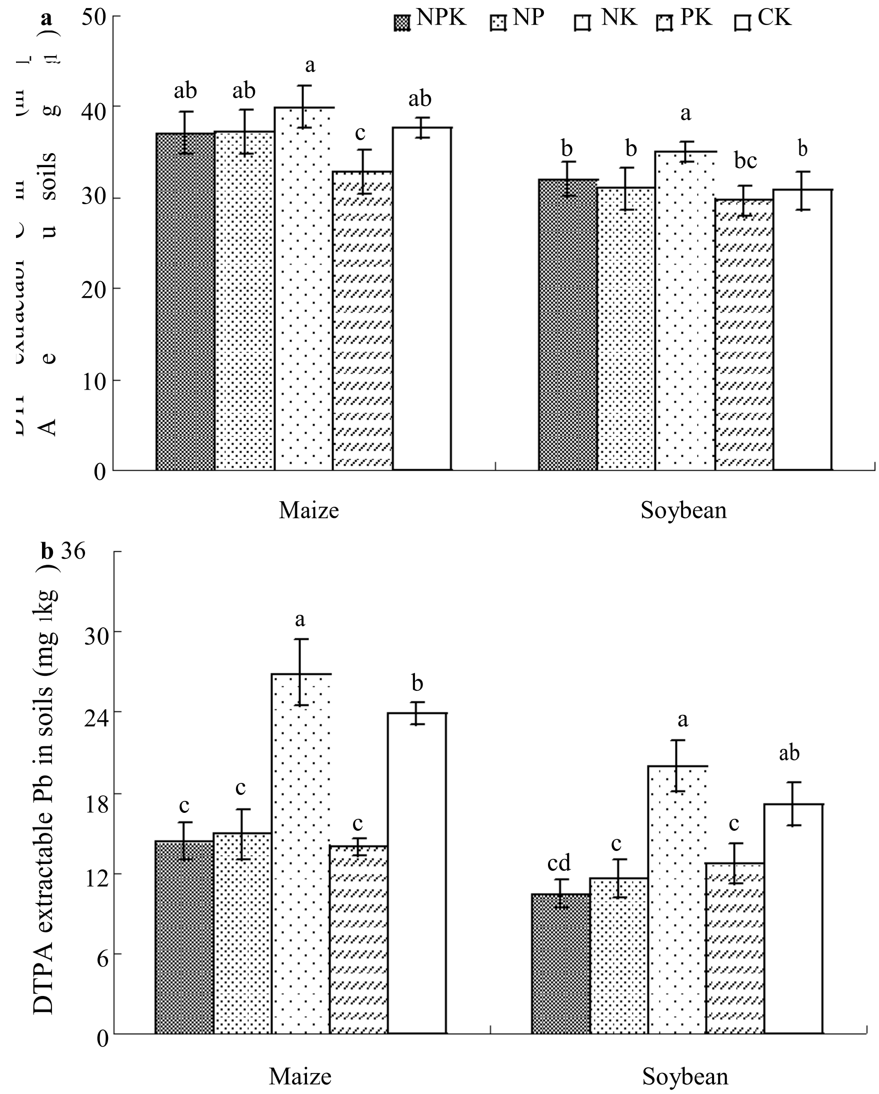

Figure 2. Soil DTPA extractable Cu and Pb showing the effect of various fertilizer treatments in a controlled greenhouse experiment. (a) DTPA extractable Cu; (b) DTPA extractable Pb. NPK, N, P, and K application; NP, N and P application; NK, N and K application; PK, P and K application; CK, no fertilization. Columns are means (n=3) and the bar on each column is ± standard deviation; means followed by the same letters within each group are not different at p<0.05.

and 4). Soil pH negatively correlated with DTPA-extractable Cu and Pb, and with DTPA-extractable Cu reaching the significant level in soil-maize system (Table 3). After P application, soil DTPA-extractable Pb decreased by 44.7~48.0% in soil-maize system and 26.2~41.8% in soil-soybean system. Previously, P-induced Pb immobilization in soil had been widely reported, which was achieved primarily through precipitation of pyromorphite in the P-fertilized treatments (Ma et al., 1993; Ma and Rao, 1997).

In accordance with Pb mobilization, S/R, root and shoot concentrations of Pb in both crops significantly increased in the treatments without P application (NK and CK). Compared to the treatments without N application, maize shoot Cu concentration and S/R of Cu significantly increased in the N-fertilized treatments (except for S/R of Cu in the NK treatment), which suggested that, N application enhance Cu translocation from soil to above ground tissue. The complexes of Cu formed with nitrogenous organic compounds (e.g. amino acids) in the plant may influence the concentration and behavior of Cu, involving Cu transportation from root to shoot (Jarvis and Whitehead, 1981). Zhou et al. (2007) also had proved that, cysteine can significantly increase Cu uptake and translocation from root to shoot by maize seedlings. But, the insignificant difference in soybean shoot Cu concentration was observed between N-fertilized treatments and treatments without N application, except

Table 3. Correlation coefficients between soil-maize interface properties and Cu and Pb accumulation.

Soil-maize interface properties [a]	Cu and Pb Accumulation [b]									
	DTPA-Cu	DTPA-Pb	Root-Cu	Root-Pb	Shoot-Cu	Shoot-Pb	Cu-S/R	Pb-S/R	T-Cu	T-Pb
AN	0.549*	0.224	0.582*	0.020	0.818**	-0.280	0.676**	-0.239	0.115	-0.249
AP	-0.681**	-0.806**	0.024	-0.720**	-0.071	-0.752**	-0.139	-0.697*	0.687**	0.003
AK	-0.119	-0.198	0.222	0.101	0.296	-0.440	0.230	-0.476	0.418	0.362
DOC	0.607*	0.681**	0.188	0.545*	0.309	0.555*	0.277	0.675**	-0.285	0.144
pH	-0.734**	-0.384	-0.425	-0.255	-0.798**	-0.023	-0.750**	0.034	-0.025	0.040
Biomass	-0.304	-0.797**	0.037	-0.534*	0.517*	-0.878**	0.581*	-0.773**	0.886**	0.365

[a] AN, available N concentration; AP, available P concentration; AK, available K concentration; DOC, soil dissolved organic C content; Biomass, dry weight of the whole maize plant. [b] DTPA-Cu (Pb), soil DTPA-extractable Cu (Pb) concentration; Root Cu (Pb), root Cu (Pb) concentration; Shoot Cu (Pb), shoot Cu (Pb) concentration; Cu (Pb)-S/R, the ratio of shoot Cu (Pb) concentration to root Cu (Pb) concentration; T- Cu (Pb), Cu or Pb accumulation in whole maize plant. * Correlation is significant at $p<0.05$; ** correlation is significant at $p<0.01$.

Table 4. Correlation coefficients between soil-soybean interface properties and Cu and Pb accumulation.

Soil-soybean interface properties [a]	Cu and Pb Accumulation [b]									
	DTPA-Cu	DTPA-Pb	Root-Cu	Root-Pb	Shoot-Cu	Shoot-Pb	Cu-S/R	Pb-S/R	T-Cu	T-Pb
AN	0.239	0.098	0.798**	0.230	0.539*	0.135	0.165	-0.160	0.476	0.050
AP	-0.053	-0.735**	-0.205	-0.700**	-0.591*	-0.827**	-0.631*	-0.557*	0.073	-0.835**
AK	0.204	0.101	0.354	0.248	0.484	0.209	0.359	0.040	0.506	0.178
DOC	0.498	0.510	0.468	0.335	0.671*	0.392	0.554*	0.181	0.148	0.296
pH	-0.312	-0.196	-0.691**	-0.354	0.498	-0.330	-0.029	-0.190	-0.500	-0.223
Biomass	-0.197	-0.721*	-0.301	-0.394	-0.685*	0.521*	-0.364	-0.711**	0.315	-0.342

[a] AN, available N concentration; AP, available P concentration; AK, available K concentration; DOC, soil dissolved organic C content; Biomass, dry weight of the whole maize plant. [b] DTPA-Cu (Pb), soil DTPA-extractable Cu (Pb) concentration; Root Cu (Pb), root Cu (Pb) concentration; Shoot Cu (Pb), shoot Cu (Pb) concentration; Cu (Pb)-S/R, the ratio of shoot Cu (Pb) concentration to root Cu (Pb) concentration; T- Cu (Pb), Cu or Pb accumulation in whole soybean plant. *Correlation is significant at $p < 0.05$; ** correlation is significant at $p<0.01$.

for the NK treatment. Correlation analysis showed that, soil available N significantly positive, correlated with root and shoot Cu concentrations in both crops (Tables 3 and 4). Meanwhile, it also positively correlated with S/R of Cu, and reached the significant level in soil-maize system.

Therefore, in our views, such differences in plant species were owing to N-fixing by soybean, which caused N application to no longer play the important role in the formation of nitrogenous organic compounds in plant. So, N-fixing by legume crops not only can facilitate plant growth, but also increase Cu uptake and translocation in soil-plant system. In addition, high available N accumulation (NK) in soil-soybean system could lead to DOC increase, which in turn enhanced Cu and Pb uptake and translocation from soil to above ground tissues (Table 2). A similar report about N accumulation on soybean growing was also reported by Zhang et al. (2007). More attention should be taken in future studies. No significant correlation was observed between K application and Cu and Pb transformation in both soil-plant systems.

Heavy metal removal efficient by plant was both relative to biomass production and tissue concentration. For both crops, combined application of N and P increased plant biomass production, which caused the increment of Cu removal from soil significantly, though the greatest soybean tissue Cu concentration being in NK treatment (Table 2). Due to Pb immobilization by P application, P application could significantly reduce Pb removal from soil by soybean (Tables 2 and 4). But, in soil-maize system, Pb removal from soil positively correlated with plant biomass production (Table 3), and the greatest was in the NPK treatment with the highest plant dry weight.

In a word, the response of plant growth to nutrients supply varied in species, which further resulted in the different Cu and Pb accumulation in plants. The present study demonstrated that, N and P application had significant effect on Cu and Pb transformation in soil-plant systems. Nitrogen application significantly increased Cu uptake and translocation in maize. N-fixing by soybean not only can facilitate plant growth, but also increase Cu uptake and translocation. Under nutrients deficiency, soil DOC increased significantly showing difference in plant species, which further boosted Cu and Pb mobility in soils. In agricultural practice, an appropriate increase in P application and decrease in N application are recommended to decrease agro-ecological risks associated with Cu and Pb. In addition, plant dry biomass increase is an efficient way to improve Cu removal from soil through fertilization.

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