

Influence of N, P, and K application on *Zea mays* L. growth and Cu and Pb accumulation

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ABSTRACT

Fertilization affects soil processes in many ways that remain unclear. The effects of N, P, and K application on plant growth and Cu and Pb accumulation were thus evaluated in a soil-maize system using five treatments: N, P, and K application; N and P application; N and K application; P and K application; and control (no fertilization). Compared to the control, fertilizer application treatments, especially N application, significantly increased maize photosynthetic rate, which further improved shoot biomass production. Root growth, root and shoot Cu and Pb concentrations, and Cu and Pb mobility also significantly altered with fertilizer application. Shoot Cu contents, root Cu uptake and translocation factors were significantly increased in the N-fertilized treatments. The significant decrease of shoot Pb concentration and root Pb uptake and increase of Cu and Pb immobilization were observed in the P-fertilized treatments. No significant correlation was observed between K application and Cu and Pb accumulation in maize. Our results show that an increase in P application and decrease in N application is recommended to reduce agro-ecological risks associated with Cu and Pb in soil-maize systems. However, the mechanisms governing the relationship between nutrients and heavy metal transformation in soil-plant systems needs further research.

Keywords: soil heavy metals; Cu and Pb mobility; ecological risk; translocation factor

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. At present, remediation technologies for soils contaminated with heavy metals are of particular interest, and fertilization is an important measure to enhance remediation efficiency and reduce ecological risks (Salah and Barrington 2006, Lin et al. 2010). The transformation of heavy metals is influenced by soil composition, physicochemical and biological properties, as well as physicochemical-biological interfacial interactions (Violante et al. 2007, 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 plant

growth and soil-plant interactions. 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 proven that dissolved soil organic matter (OM) have significant effects on the transformation of heavy metals in soil-plant systems, mainly through incremental heavy metal solubility, plant uptake, and translocation (Wong et al. 2007, Quartacci et al. 2009, Kim et al. 2010). Plant transpiration, photosynthesis, amino acid metabolism, and biomass production, which all directly or indirectly regulate the behaviors of heavy metals in soil-plant systems (Jarvis and Whitehead 1981, Ruley et al. 2006, Salah and Barrington 2006), are also affected by N, P, and K application. On the other hand, the bioavailability of heavy metals varied in soils with

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different fertilization management owing to the differences in soil pH, OM percentage, microbial activity, and heavy metal mobilization (Ma et al. 1993, Miller 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). To date, however, the effect of nutrient application on the behavior of heavy metals in soil-plant systems, such as heavy metal availability, plant uptake, and translocation, is still not fully understood.

Copper and lead are two of the most widely reported heavy metal soil pollutants in the world (Jensen-Spaulding et al. 2004, Takáč et al. 2009). In the present study, their accumulation in maize (*Zea mays* L.) was investigated under different N, P, and K application schemes. Our objectives were to evaluate the effects of various nutrient application schemes on plant growth and soil-plant interactions as well as Cu and Pb accumulation in maize, with a special focus on the optimization of nutrient management for decreasing ecological risks from heavy metal pollution.

MATERIAL AND METHODS

Soil used. The soil, classified as an Aquic Inceptisol with a sandy loam, was collected from topsoil (0–20 cm) in an agricultural field. It was air-dried, ground and sieved (< 2 mm). In the pretreated soil, organic C and total N and P concentrations were 8.82, 0.45, and 0.41 g/kg, respectively. Soil available N, P, and K concentrations were 10.85, 6.50 and 46.58 mg/kg, respectively, and pH was 7.5. Total Cu and Pb concentrations were 14.5 and 21.9 mg/kg, respectively. Chemical analysis methods used to acquire pretreated soil properties are given below.

Experimental design. The soil used in this study was amended with Cu^{2+} (100 mg/kg, $\text{CuCl}_2 \cdot 2 \text{H}_2\text{O}$) and Pb^{2+} (100 mg/kg, 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 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 rins-

ing with deionized water, the germinated seeds of Taiyu-2 maize (*Zea mays* L.) cultivar were sown in the pots. The seedlings were subsequently thinned to 1 plant per pot after emergence and grown under greenhouse conditions ranging from 20 to 30°C during the day and 15 to 20°C at night. Inorganic salt solution was applied weekly as the nutrient source for plants, which involved five treatments in triplicate, i.e., NPK (80 N mg/kg, 35 P mg/kg, and 60 K mg/kg), NP (80 N mg/kg, and 35 P mg/kg), NK (80 N mg/kg, and 60 K mg/kg), PK (35 P mg/kg, and 60 K mg/kg) and control (deionized water only). To avoid water leaching from pots, 300 ml of inorganic salt solution was 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, roots and shoots were harvested and separated from the soil, washed with running water, and rinsed thoroughly with deionized water. Using WinRHIZO, root length and surface area were determined. Before harvest, maize photosynthesis rate (P_n) and transpiration rate (T_r) were measured using a portable photosynthesis system (LI-6400 XT, Lincoln, Nebraska, USA). The shoot was dried at 60°C for 48 h, and the root was divided into two aliquots. One was dried in the same manner as the shoots, and the other was washed with an EDTA solution and then dried as above. The dry biomass was 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 and root samples were digested with $\text{HNO}_3\text{-HClO}_4\text{-H}_2\text{SO}_4$ (7:2:1, v/v) to determine 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). Root Cu and Pb accumulation included that in apoplast and symplast. Copper and lead remaining in EDTA-washed roots were considered to be in symplast, expressed as root metal uptake, and heavy metal concentrations in unwashed roots

were considered to be the metal accumulation in roots (Zhou et al. 2007).

Soil analysis. Soil pH was measured in a soil-water suspension (1:2.5 v/v). Total soil organic C and N concentrations were determined with a vario EL III analyzer (Elementar, Hanau, Germany). Available N was extracted with 2 mol/L KCl and analyzed with a segmented flow analyzer (Skalar, Netherlands). Available P and available K were extracted by sodium bicarbonate and ammonium acetate, respectively (Lu 2000). Dissolved organic C was extracted with ultrapure water and measured by a liquiTOC II analyzer (Elementar, Hanau, Germany) after filtration through a 0.45 µm milipore filter (Martín-Olmedo and Rees 1999).

Statistical analysis. A heavy metal translocation factor (TF) was calculated as the ratio of the shoot Cu or Pb concentration to the root Cu or Pb uptake. All data are mean values from three replicates. Using SPSS software (12.0), the significant differences between means were determined by *LSD* at $P < 0.05$.

RESULTS

Soil properties. Soil properties are shown in Table 1. The lowest soil pH was observed in the NK treatment, which was significantly lower than other four treatments, followed by the NPK and NP treatments, and the greatest in the PK treatment. The greatest soil organic C was in the NPK treatment, which was significantly higher than that in the NK treatment. Soil dissolved organic C (DOC) was significantly higher in the treatments without P application than that in the P-fertilized treatments except for the NPK treatment, with the control treatment showing the highest. Total soil N, available N, P, and K concentrations were significantly higher in the treatments where N, P, or K was applied.

Maize growth and biomass production. Maize growth was significantly influenced by fertilizer application (Table 2). Compared to the control, maize shoot dry weight was significantly greater in the fertilized treatments: the highest weight was in the NPK and NP treatments, followed by the PK treatment. For maize roots, however, the greatest to least dry weights per treatment were NPK > PK > NP > control > NK. Root length and surface area were significantly lower in the NK and control treatments than those in the NPK, NP, and PK treatments. Maize photosynthetic rate and transpiration rate were significantly higher in the N-fertilized treatments with the control treatment showing the least.

Cu and Pb accumulation in maize. The shoot Cu concentrations in the N-fertilized treatments were significantly higher than those in the treatments without N application, which were about twice as high as in the PK treatment. The greatest Cu concentration was found in the NP treatment, followed by NPK and NK treatments. After P application, shoot Pb concentrations significantly decreased, and the greatest was in the control, followed by the NK treatment (Table 3).

Root Cu and Pb concentrations were far higher than those in shoots. Copper accumulation in roots ranged from 243.7 to 302.8 mg/kg, and Pb accumulation from 56.1 to 76.1 mg/kg. The greatest root Cu and Pb accumulations were both in the NK treatment, but the increase was insignificant compared to the other four treatments except for root Pb accumulation in the NP treatment (Table 3). Compared to treatments without N application, root Cu uptake was significantly higher in the N-fertilized treatments. The TF of Cu showed similar trends as root Cu uptake. Compared to the P-fertilized treatments, root Pb uptake significantly increased in the treatments without P application, however TF of Pb among the five treatments was insignificant (Table 3).

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

Fertilizer application treatments	pH (H ₂ O)	SOC	Total N	DOC	Available N	Available P	Available K
		(g/kg)		(mg/kg)			
NPK	7.24 ^c	9.49 ^a	0.49 ^a	17.15 ^b	54.15 ^c	23.98 ^b	124.44 ^a
NP	7.31 ^c	9.41 ^{ab}	0.48 ^a	16.02 ^{bc}	83.45 ^b	31.92 ^a	54.72 ^b
NK	7.15 ^d	8.99 ^b	0.51 ^a	17.37 ^{ab}	114.17 ^a	9.42 ^c	92.53 ^{ab}
PK	7.80 ^a	9.19 ^{ab}	0.40 ^b	15.06 ^c	2.40 ^d	35.30 ^a	66.64 ^b
Control	7.44 ^b	9.11 ^{ab}	0.40 ^b	18.77 ^a	2.65 ^d	7.71 ^c	20.59 ^c

SOC – soil organic C; DOC – soil dissolved organic C; Means ($n = 3$) with the same letter within columns are not significantly different at $P < 0.05$

Table 2. Maize growth showing the effect of various fertilizer treatments in a controlled greenhouse experiment

Fertilizer application treatments	Shoot	Root	Root length	Root surface area	P_n	T_r
	(g)	(g)	(cm)	(cm ²)	($\mu\text{mol}/\text{m}^2/\text{s}$)	($\mu\text{mol}/\text{m}^2/\text{s}$)
NPK	2.22 ^a	0.61 ^a	280.8 ^a	52.8 ^a	19.20 ^a	1.93 ^a
NP	2.25 ^a	0.54 ^b	223.9 ^b	44.2 ^{bc}	17.34 ^{ab}	2.01 ^a
NK	1.42 ^b	0.34 ^d	158.5 ^c	34.7 ^d	15.91 ^b	1.72 ^b
PK	1.54 ^b	0.59 ^b	241.6 ^b	46.2 ^{ab}	12.09 ^c	1.31 ^c
Control	1.07 ^c	0.46 ^c	165.2 ^c	37.0 ^{cd}	8.47 ^d	1.17 ^d

Shoot – dry shoot weight; Root – dry root weight; P_n – photosynthetic rate; T_r – transpiration rate. Means ($n = 3$) with the same letter within columns are not significantly different at $P < 0.05$

DTPA extractable metals in soils. The highest soil DTPA-extractable Cu concentration were observed in the NK treatment, followed by the control, and the least in the PK treatment which was significantly lower than that in other four treatments (Figure 1). After addition of P, DTPA-extractable Pb concentrations significantly decreased, and the greatest was also found in the NK treatment, which was significantly higher than that in the other four treatments.

DISCUSSION

Effect of NPK on soil properties and maize growth. It is no doubt that soil N, P, and K concentration increase with N, P, and K application. DOC was mainly from root exudates and decomposition products in soil (Jones 1998). High DOC in the NK and control treatments may be from the

enhancement of root exudates under P deficiency (Zhang et al. 1997). Unbalanced N fertilization can lead to soil pH decrease (Darilek et al. 2009, Guo et al. 2010), and the NK treatment with the greatest N accumulation had 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 maize photosynthetic rate, which further improved shoot production (Table 2). However, due to unbalanced N fertilization, soil available N concentration increased greatly (Table 1), which negatively influenced root biomass production (Li et al. 2009), and root growth was significantly inhibited in the NK treatment, showing the least dry root weight, root length, and surface area.

Effect of NPK on Cu and Pb accumulation in maize. The mobilization of heavy metals positively

Table 3. Cu and Pb accumulation and translocation factors showing the effect of various fertilizer treatments in a controlled greenhouse experiment

Heavy metal property		Fertilizer application treatments				
		NPK	NP	NK	PK	Control
Shoot (mg/kg)	Cu	16.10 ^{ab}	17.80 ^a	14.99 ^b	7.73 ^d	10.61 ^c
	Pb	5.30 ^c	4.77 ^c	7.99 ^b	5.79 ^c	9.86 ^a
Root (mg/kg)	Cu	277.2 ^a	279.4 ^a	302.8 ^a	248.4 ^a	243.7 ^a
	Pb	66.3 ^{ab}	56.1 ^b	76.1 ^a	61.9 ^{ab}	74.7 ^a
EDTA-root (mg/kg)	Cu	100.1 ^{ab}	109.0 ^a	95.2 ^b	79.8 ^c	82.5 ^c
	Pb	13.7 ^b	12.0 ^b	19.7 ^a	14.0 ^b	22.5 ^a
TF (%)	Cu	16.4 ^a	15.1 ^{ab}	15.8 ^{ab}	9.8 ^d	12.9 ^c
	Pb	40.2 ^a	39.8 ^a	40.7 ^a	42.0 ^a	43.8 ^a

EDTA-root – Cu or Pb remaining in EDTA-washed roots, which was considered to be in symplast and expressed as root metal uptake; TF – translocation factor, which was expressed as the ratio of the shoot Cu or Pb concentration to the root Cu or Pb uptake. Means ($n = 3$) with the same letter within columns are not significantly different at $P < 0.05$

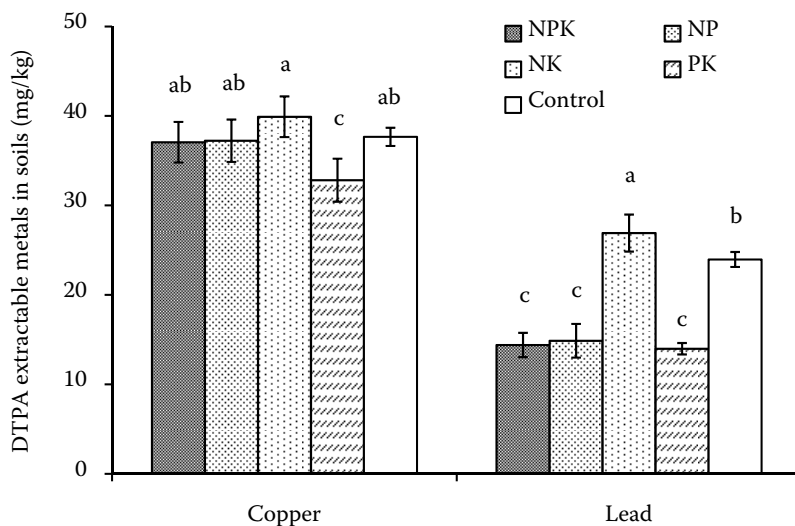


Figure 1. DTPA extractable Cu and Pb showing the effect of various fertilizer treatments in a controlled greenhouse experiment. Columns represent means ($n = 3$) and bars on each column represent standard deviation; means with the same letter within groups are not different at $P < 0.05$

correlates with their DTPA-extractable amount in soil (Rajkumar et al. 2010). Before maize was sown, DTPA-extractable Cu and Pb concentrations were 34.3 mg/kg, and 25.7 mg/kg, respectively, in contaminated soil. DTPA-extractable Cu concentrations were significantly lower than that in sown soils except for the PK treatment, while DTPA-extractable Pb was significantly higher than that in the P-fertilized treatments. This suggests that chemical fertilizer application and plant growth may influence Cu and Pb mobilization in soil (Martínez-Alcalá et al. 2010).

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. In the present study, correlation analysis indicated that DOC was significantly positively correlated with DTPA Cu and Pb ($r = 0.607, 0.681$, respectively; $P < 0.05$). With increasing DOC, higher soil DTPA-extractable Cu and Pb concentrations were observed in the control and NK treatments. Soil available P concentrations were significantly negative with Cu and Pb mobility ($r = -0.681, -0.806$, respectively; $P < 0.05$). Previously, P-induced Pb immobilization in soil was widely reported, which was achieved primarily through precipitation of pyromorphite in the P-fertilized treatments (Ma et al. 1993, Ma and Rao 1997). The least DTPA-extractable Cu concentrations in the PK treatment indicated that P application could also effectively increase Cu immobilization in soil, mainly through increasing pH (Pérez-Novo et al. 2009).

In accordance with Pb mobilization, root Pb uptake and shoot Pb concentrations both signifi-

cantly increased in the NK and control treatments. However, root Cu uptake, shoot Cu concentrations and TF of Cu were all significantly higher in the N-fertilized treatments compared to those in the treatments without N application, and available N concentrations were positively correlated with shoot Cu concentrations, root Cu uptake, and TF of Cu ($r = 0.818, 0.682, 0.676$, respectively; $P < 0.05$). Many studies indicate that Cu (II) may be reduced to Cu (I) prior to its absorption into root cells and transport in plants (Wintz and Vulpe 2002, Zheng et al. 2005). Nitrogen application improved the production of nitrogenous organic compounds (e.g. amino acids) in the plant (Jarvis and Whitehead 1981), which could facilitate Cu (II) reduction and translocation. Zhou et al. (2007) proved that cysteine can significantly increase Cu uptake and translocation from root to shoot by maize seedlings. Although Pb immobilization due to the presence of P lead to significant reduction in maize Pb uptake, and soil available P concentrations were negatively correlated with shoot Pb concentrations and root Pb uptake ($r = -0.752, -0.780$, respectively; $P < 0.05$), TF of Pb did not significantly decrease in the P-fertilized treatments. Therefore, P supply had little effect on Pb translocation from root to shoot. Also, no significant correlation was observed between K application and Cu and Pb accumulation in maize.

Because of the complexity of heavy metal transformation in soil-plant systems, some factors influencing the behavior of Cu and Pb might be lost in this study, such as maize transpiration rate (Salah and Barrington 2006). Even so, the findings of the present study have important implications

for nutrient management of crop production in heavy metal polluted areas. To enhance crop yield, mineral N is increasingly being applied, while P fertilizer application is either underestimated or ignored (Xie et al. 2008). This practice can lead to soil pH decrease and increasing DOC, which will further increase heavy metal mobilization and plant uptake. Therefore, to decrease agro-ecological risks associated with Cu and Pb, an appropriate increase in P application and decrease in N application is recommended. Further investigation is required to give more mechanistic evidences to elucidate the relationship between nutrient supply and heavy metal uptake and translocation in future studies.

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