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Nutrients can enhance phytoremediation of copper-polluted soil by Indian mustard

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Abstract

An orthogonally designed experiment was conducted to study the single and combined effects of N, P and K on phytoremediation of Cu-polluted soil by Indian mustard. Addition of fertilizer N and P significantly increased Indian mustard shoot yield. Two high treatments combined with N resulted in the highest yields, followed by low-P combined with N. In contrast, high P with no N gave no yield increase and K had no effect on yield of Indian mustard. Nitrogen and P increased the amount of chlorophyll in the leaves, indicating that the yield increases were due to enhanced photosynthesis. Nitrogen application had no effect on plant Cu concentrations but addition of P slightly decreased plant Cu concentrations, likely a dilution effect resulting from the increase in yield. Among the treatments, N and P applied at 100 and 200 mg kg⁻¹ respectively with no K application resulted in the highest Cu uptake. Thus, a combination of low N and high P produced a yield increase in Indian mustard that was more than adequate to compensate for a slight decrease in Cu concentration, resulting in the highest Cu removal from the contaminated soil.

1. Introduction

Soil ecosystems throughout the world have been contaminated with heavy metals by various human activities, and movement of metals up the food chain has become a human health hazard (Tu *et al.* 2000; Dahmani-Muller *et al.* 2001; Khan 2001; Qiao & Luo 2001; McGrath *et al.* 2002). One of the most promising and potentially cost-effective soil remediation techniques is phytoremediation (Ebbs & Kochian, 1997; Huang *et al.* 1997; Blaylock 2000). The ideal plant species to remediate a heavy metal contaminated soil would be a high biomass crop that can both tolerate and accumulate the contaminants of interest. At present only a small number of higher plant species which can survive in metal-polluted soils and extract and translocate metals from roots to the

above-ground parts are used in phytoremediation technology (Brooks 1998).

Indian mustard (*Brassica juncea*) has shown good potential for phytoremediation of heavy metal contaminated soil. However, one drawback is that the plants often need specific nutrients, and there has been little research on nutrient enhancement of plant metal uptake (Zaurov *et al.* 1999). The present study was undertaken to examine the effects of the major nutrients N, P and K singly or in combination on phytoremediation by Indian mustard of Cu-polluted soil. In the present study, shoot dry matter yield and chlorophyll content of leaves together with Cu accumulation in the shoots of Indian mustard were used to determine whether nutrient enhancement could be used to stimulate plant growth and thus Cu uptake from a soil contaminated with Cu.

2. Materials and methods

2.1. Experimental design and growth media

Experiments were set up in a glasshouse at the Institute of Soil Science, Chinese Academy of Sciences, Nanjing. Soil was collected from Fengyi town, Yixing city, Jiangsu province, in 1998. The soil had been polluted by irrigation with waste-water contaminated with CuSO_4 discharged from a Cu smelter in 1997. Soil pH (1:2.5 soil:water ratio) was 6.3, and soil total Cu was 70 mg kg^{-1} . The soil organic carbon content, CEC were 16.5 g kg^{-1} , $19.0 \text{ cmol kg}^{-1}$, respectively. Soil total N, P and K were 1.51, 0.83 and 15.7 g kg^{-1} , respectively. And the soil available N, Olsen P and exchangeable K were 160, 9.07 and 103 mg kg^{-1} , respectively. Air-dried <2 mm soil equivalent to 350 g (oven dry basis) was placed in each plastic pot (8.0 cm diameter and 8.0 cm high).

An orthogonal design (9L_3^4) was adopted (North-east Agricultural University, South China Agricultural University, 1980), and the fourth row was empty (Table 1). Fertilizer N was supplied as urea (46.7% N) at 200 and 400 mg kg^{-1} , P was supplied as superphosphate (18% P_2O_5) at 100 and 200 mg kg^{-1} and K was supplied as KCl (52.3% K_2O) at 100 and 200 mg kg^{-1} . All the P and half of the N and K were added as basal applications, and the remaining N and K were applied in two subsequent applications one and two months after the seeds were sown.

Indian mustard (*Brassica juncea* L.) was sown at the rate of three seeds per pot. Ten days after germination one seedling was retained in each pot. Deionised water was added daily to bring the pots

to 70% soil water-holding capacity by weight. The plants grew from October to December in 2001 at a temperature of $18 \pm 3^\circ\text{C}$. There were four replicates of each treatment arranged in a replicate block design.

2.2. Sampling and analysis

Plants were harvested after 80 days' growth and rinsed with deionized water. Shoots were separated from roots. Dry matter yields of shoots were recorded after drying at 80°C for 2 days to constant weight. After digestion of ground plant samples (0.5 g each) with $\text{HNO}_3\text{--HClO}_4$ (3:2), Cu was measured by flame atomic absorption spectrophotometry (FAAS). Soil pH (soil:water ratio 1:2.5) was measured using a pH meter. Soil exchangeable Cu (extracted with NH_4OAc at pH 7.0) and total Cu (digested with $\text{HNO}_3\text{--HClO}_4\text{--HF}$) were measured by FAAS. Data presented are means of four replicates together with standard errors of the means (SEM), and was analyzed following Tang and Feng's method (Tang & Feng 2002).

3. Results and discussion

3.1. Shoot yields

As shown in Figure 1, shoot yield increased with the addition of N ($p < 0.01$), but there was no difference between low N and high N addition ($p > 0.05$). The influence of the three P treatments on shoot yield gave a highly significantly ($p < 0.01$) positive correlation, while no significant influence of different K addition levels on yield was found (Table 2). The results show that there was a marked interactive effect of fertilizers on shoot yield. The greatest yields occurred when $<200 \text{ mg P kg}^{-1}$ was combined with N application (treatments 9 and 6), followed by intermediate yields with $<100 \text{ mg P kg}^{-1}$ combined with N application (treatments 5 and 8) and lowest yields when 200 mg P kg^{-1} was applied with no N application (treatment 3). These results suggest that P supply was the primary factor influencing Indian mustard yield, and when P was combined with N application, effect of the interaction between N and P was greater than that of N and P supplied singly.

Table 1. Treatments of the orthogonal design with 4 factors and 3 levels.

Treatment	1 N	2 P	3 K	4 Blank
1 $\text{N}_0\text{P}_0\text{K}_0$	1	1	1	1
2 $\text{N}_0\text{P}_1\text{K}_1$	1	2	2	2
3 $\text{N}_0\text{P}_2\text{K}_2$	1	3	3	3
4 $\text{N}_1\text{P}_0\text{K}_1$	2	1	2	3
5 $\text{N}_1\text{P}_1\text{K}_2$	2	2	3	1
6 $\text{N}_1\text{P}_2\text{K}_0$	2	3	1	2
7 $\text{N}_2\text{P}_0\text{K}_2$	3	1	3	2
8 $\text{N}_2\text{P}_1\text{K}_0$	3	2	1	3
9 $\text{N}_2\text{P}_2\text{K}_1$	3	3	2	1

Fig. 1. Dry matter yields of Indian mustard shoots (mg pot^{-1}). For key to treatments, see Table 1. Bars: standard error of the mean (SEM).

3.2. Chlorophyll content

Leaf chlorophyll content increased significantly with application of N fertilizer ($p < 0.01$), but there was no difference between the 200 and 400 mg N kg^{-1} application rates (Figure 2). The

increase in leaf chlorophyll content due to application of P fertilizer was significant ($p < 0.01$) (Table 2). The interactive effect of the fertilizer nutrients on chlorophyll content was similar to that on shoot yield, suggesting that P combined with N application may be important for Indian mustard growth in this soil. Moreover, when N and P were applied in combination, K application had only a slight effect on chlorophyll content. Two likely explanations for this are: (1) an effect of unbalanced fertilizer nutrient supply on physiological processes, and (2) some influence by the different fertilizer combinations on the uptake of other nutrient elements by the plants.

3.3. Shoot copper concentration

Shoot Cu concentrations are shown in Figure 3. Compared to the other P treatments, Cu concentrations in the plants decreased under the highest P treatment, and no difference was found between 100 and 0 mg P kg^{-1} . However, compared with 100 mg P kg^{-1} , the effect of 200 mg P kg^{-1} on Cu

Table 2. Statistical results of N, P and K on plant dry weight, chlorophyll content, Cu concentration and Cu uptake of Indian mustard ($n = 12$)^a.

Treatment (mg kg^{-1} soil)	Dry Weight (mg pot^{-1})	Chlorophyll content (mg g^{-1} fresh weight)	Cu concentration (mg kg^{-1})	Cu uptake ($\mu\text{g pot}^{-1}$)
N				
0	1.17 ± 0.04	353.8 ± 10.2	13.4 ± 3.8	14.0 ± 1.9
200	1.91 ± 0.25	421.4 ± 5.2	17.4 ± 6.2	26.7 ± 2.0
400	1.92 ± 0.14	417.3 ± 11.3	15.2 ± 2.9	23.2 ± 1.3
LSD ($p = 0.05$) ^b	0.51	28.7	7.87	5.39
Significance ^c	**	**	n.s.	**
P₂O₅				
0	0.76 ± 0.12	368.7 ± 15.9	21.1 ± 5.6	12.2 ± 1.4
100	1.87 ± 0.06	394.8 ± 7.9	15.1 ± 2.7	25.4 ± 3.8
200	2.38 ± 0.06	429.0 ± 10.4	9.74 ± 2.7	26.2 ± 3.8
LSD ($p = 0.05$) ^b	0.344	36.7	6.73	10.0
Significance ^c	**	**	**	**
K₂O				
0	1.76 ± 0.10	447.1 ± 13.4	16.3 ± 2.1	27.9 ± 3.2
100	1.63 ± 0.09	395.3 ± 8.5	16.6 ± 6.1	19.7 ± 1.3
200	1.61 ± 0.14	350.1 ± 5.6	13.1 ± 4.5	16.3 ± 1.7
LSD ($p = 0.05$) ^b	0.291	30.1	6.24	6.95
Significance ^c	n.s.	**	n.s.	*

^a For key to treatments, see Table 1.

^b Least significant difference at the 5% level.

^c By analysis of variance: * $p < 0.05$; ** $p < 0.01$; n.s., not significant.

Fig. 2. Chlorophyll content in leaves of Indian mustard (mg g^{-1} fresh weight). For key to treatments, see Table 1. Bars: SEM.

Fig. 3. Cu concentration in Indian mustard shoots (mg kg^{-1}). For key to treatments, see Table 1. Bars: SEM.

concentration was significant ($p < 0.01$) (Table 2). The greatest shoot Cu concentrations occurred at 0 mg P kg^{-1} (treatments 1 and 7), and it was decreased with the added P fertilizer, which may result from a dilution effect of the increase in shoot yield.

There were no significant treatment effects on soil exchangeable Cu (Table 3). Soil pH responded only the acidifying effect of urea-N.

3.4. Relationship between enhanced nutrient uptake and Cu uptake

Shoot Cu uptake was significantly ($p < 0.01$) affected by the low level of N application (100 mg N kg^{-1}) and increased with increasing N application rate (Figure 4, Table 2). K fertilizer significantly ($p < 0.05$) affected the plant Cu uptake at the low level ($100 \text{ mg K}_2\text{O kg}^{-1}$), but there was no difference between the K dosages. Com-

Table 3. Soil NH_4OAc exchangeable Cu and pH of the orthogonally designed experiment^a.

Treatment	NH_4OAc exchangeable Cu (mg kg^{-1})	pH (H_2O)
1 $\text{N}_0\text{P}_0\text{K}_0$	1.60 ± 0.14	5.13 ± 0.08
2 $\text{N}_0\text{P}_1\text{K}_1$	1.42 ± 0.12	5.28 ± 0.12
3 $\text{N}_0\text{P}_2\text{K}_2$	1.57 ± 0.12	5.20 ± 0.14
4 $\text{N}_1\text{P}_0\text{K}_1$	1.43 ± 0.22	4.68 ± 0.04
5 $\text{N}_1\text{P}_1\text{K}_2$	1.54 ± 0.02	4.83 ± 0.12
6 $\text{N}_1\text{P}_2\text{K}_0$	1.59 ± 0.04	5.06 ± 0.08
7 $\text{N}_2\text{P}_0\text{K}_2$	1.60 ± 0.16	4.75 ± 0.16
8 $\text{N}_2\text{P}_1\text{K}_0$	1.59 ± 0.14	4.65 ± 0.12
9 $\text{N}_2\text{P}_2\text{K}_1$	1.60 ± 0.10	4.87 ± 0.10

^a For key to treatments, see Table 1.

Fig. 4. Cu uptake by Indian mustard shoots ($\mu\text{g pot}^{-1}$). For key to treatments, see Table 1. Bars: SEM.

pared with the other combined treatments, all the P-N interactions significantly increased shoot Cu uptake, with treatment 6 ($\text{P}_{200}\text{N}_{100}\text{K}_0$) showing the most pronounced effect. The increase in shoot DM yield resulting from fertilizer application was more than adequate to compensate for the decrease in shoot Cu concentration. Thus the greatest Cu removal (and therefore phytoremediation effectiveness) was achieved using the highest P application combined with the lowest N application rate.

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