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Accumulation of chromium by *Commelina communis* L. grown in solution with different concentrations of Cr and L-histidine*

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Abstract: Hydroponic experiments conducted to examine the chromiun uptake by *C. communis* in the presence of different Cr concentrations (Cr^{6+} 100 and 200 mg/L, respectively) and free histidine supplementation (0.5 and 1.0 mol/L) showed that shoot and root growth of *C. communis* decreased greatly with increasing Cr concentrations in the medium; and that the species was a typical excluder since it accumulated high concentrations of Cr in roots but comparatively low concentrations in shoots. Chromium in shoots and roots of Cr_4^{2-}-supplied plants ranged from 329 – 1880 and 3788 – 4240 mg/kg DW, respectively, while those of Cr_4^{2-}-histidine-supplied plants ranged from 478 to 629 mg/kg and 4157 – 4303 mg/kg DW, respectively. With Cr present in the hydroponic solution, *C. communis* accumulated more Cr in its tissues. Increasing histidine application to the solution significantly increased chromium accumulation in the plant tissues but could not alter the accumulation pattern of plants although it induced a higher concentration of Cr in its shoots and roots. These features suggested that *C. communis* may serve as an alternative species in a constructed wetland for phytoextraction treatment of Cr-containing wastewater and for phytostabilization of Cr mining spoils.

Key words: Cr concentration uptake, *C. communis*, Histidine, Bio-environmental engineering
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INTRODUCTION

C. communis is a widely distributed indicator that was found to be able to survive on mining wastes containing copper minerals such as malchite, azurite, chalcopyrite, and pyrite minerals (Tang et al., 1997; 1999). This species can also abnormally accumulate copper and other metals to a great extent in its shoots and roots (Tang et al., 1999; Tang et al., 2000). As more people become aware of the importance of geo-indicators in the green clean-up field (Baker, et al. 1981; Baker et al. 1988; Brooks et al. 1992; Reeves et al. 1995), research on them is becoming increasingly attractive. A review of literature showed that Brassicaceae family S-loving plants such as cauliflower, kale, cabbage were the most effective in uptaking chromium from culture medium (Lahouti et al., 1979; Zayed et al., 1998). This made us think that *C. communis*, an S-loving species, may possibly hyperaccumulate chromium. However, little work has been done in China to exploit the potential ability of this species to abnormally accumulate chromium. A greenhouse hydroponic culture experiment was conducted to investigate the uptake and translocation of Cr in *C. communis* so that important information obtained could be applied for phytoremediation of chromium-contaminated soils, sediments, and water, and for exploring the possible use of this indicator in environmental clean-up.

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Our objectives were: (1) to investigate whether or not *C. communis* could accumulate Cr from hydroponic solution and to what extent it can accumulate Cr; (2) whether histidine could enhance the uptake of Cr by this species.

MATERIALS AND METHODS

Experiment 1

This experiment was conducted to investigate the growth and Cr uptake responses of *C. communis* to culture solution Cr concentrations of 100 to 200 mmol/L. Seeds of *C. communis* collected from the copper mining spoils at Tongling, Anhui Province, were germinated in a mixture of pearlite and vermiculite (volume ratio 1 : 1) for 53 days. Five plants of each species were then transferred to 5-liter pots containing a continuously aerated half-strength nutrient solution adjusted to pH 6.5 using KOH. After seven days' growth, seedlings of the plant were transferred to full-strength nutrient solutions with different Cr concentrations. The nutrient solution contained (mmol/L): 3KNO_3, 2$Ca(NO_3)_2$, 0.5$MgSO_4$, 0.01 FeNa-EDTA, 1 $NH_4H_2PO_4$, 9×10^{-3} $MnSO_4$, 46×10^{-3} H_3BO_3, 50×10^{-3} KCl, 1.5×10^{-3} $CuSO_4$, 1.5×10^{-3} $ZnSO_4$, 0.14×10^{-3} $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (Baker et al., 1994). The control treatment contained the same basic nutrient solution but with no Cr added. After seven weeks' growth, one group of plants received Cr^{6+} 100 mg/L (Cr 19.2 μmol/L) and the other group of plants received Cr^{6+} 200 mg/L (Cr 38.4 μmol/L) supplied as potassium chromate, respectively. The nutrient solution was replaced weekly and the total volume of the solution was maintained by adding deionized water to compensate for water lost through evaporation and plant transpiration. Each treatment was replicated four times. All pots were put on benches in a greenhouse without climate control and at ambient temperature with natural illumination. The experiment lasted from March to July, 1999. After a growth period of 28 days following Cr application, the plants were harvested. Shoots and roots were rinsed and cleaned with deionized water. The biomass (fresh weight), the length of main roots, and the dry matter (DM, dried at 70℃ for 72 h) were measured and recorded. The plant material was then ground using an agate ball mill. Sub-samples were taken and ashed at 450℃ for over five hours. The resulting ash was digested with 1 mol/L HNO_3, and Cr concentrations in the digests were determined using Flame Atomic Absorption (Spectra 220).

Experiment 2

In this experiment we investigated the role of L-histidine in the uptake of Cr by *C. communis* from solution through the root system. Seedlings of *C. communis* were grown for 36 d in half-strength nutrient solution with the same composition as described in Experiment 1. The plants were then treated with Cr^{6+} 200 mg/L (Cr 38.4 μmol/L) supplied as potassium chromate. Meanwhile, three L-histidine treatments (0, 0.5, and 1.0 mol/L) were used. The control treatment had no L-histidine added. Each treatment was replicated in four vessels, with each vessel having five plants. The nutrient solution was renewed weekly and the total volume of the solution was maintained with deionized water to make up for the water lost through plant transpiration and evaporation. The plants were harvested after 21 days growth following histidine application. The lengths of the main roots and the growth response were measured. The plant materials were processed and analyzed for total Cr in the same way as done in Experiment 1.

RESULTS AND DISCUSSION

Plant growth

In experiment 1 the solution supporting the growth of *C. communis* contained low to high Cr concentrations. Adding Cr^{6+} to the growth medium increased the root length of *C. communis* but decreased the shoot length. With increasing Cr concentrations in the solution, the plant yields were greatly depressed (Fig. 1). All the test plants grown from high Cr concentration treatments produced much less biomass than that from the control treatments. It was also found that plants growing in solutions supplied with higher concentrations of histidine yielded less shoot DM than those treated with lower concentrations of the chemical (Fig.1), showing addition of histidine slightly depressed biomass production in *C. communis*. This effect may be due to the difference in tolerance to histidine.

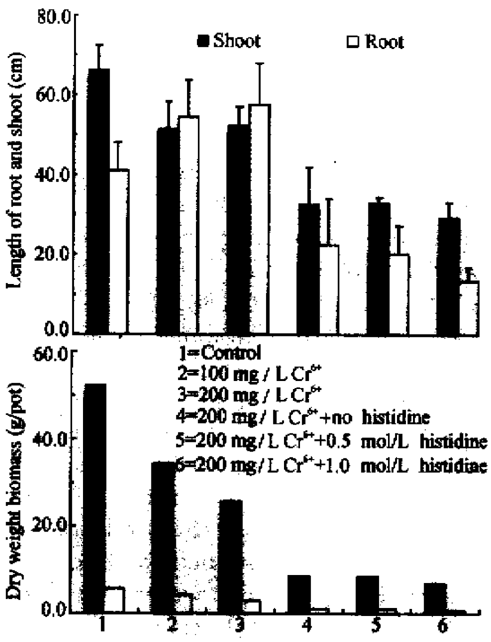

Fig.1 Length of plant tissues (upper) and biomass production (lower) of *C. communis* grown in hydroponic solution supplied with different levels of Cr^{6+} and histidine

Accumulation of chromium by *C. communis*

The ability to accumulate Cr varied to a great extent for different species (Chen et al., 1996). Our experiment showed that *C. communis* could accumulate much Cr. Chromium concentrations in roots and shoots of Cr_4^{2-} supplied plants ranged from 329 – 1880 mg/kg DW, and 3788 – 4240 mg/kg DW, respectively, while those of histidine-supplied plants ranged from 478 – 629 mg/kg DW, and 4157 – 4303 mg/kg DW, respectively. It was apparent that shoot concentration of Cr for *C. communis* mostly exceeded one-fifteenth of that in root. Regardless of histidine treatments, Cr concentrations in the shoots of *C. communis* were significantly affected by the levels of Cr in the solution (Fig.2). Accumulation of Cr in shoots and roots of *C. communis* was dependent on the Cr concentrations in the medium. With increase of Cr concentrations in the medium, the plants showed an increasing uptake of Cr in their tissues (Fig.2). More interestingly, the accumulation of Cr by *C. communis* was also related to the application of histidine application in the solutions. Both shoot and root accumulation of Cr was greatest in *C. communis* when histidine was supplied at 1.0 mol/L (Fig.3). It was apparent that histidine-supplied plants accumulated more Cr in roots and shoots than plants without external histidine supply, though differences were not statistically significant (Fig.3). It appeared

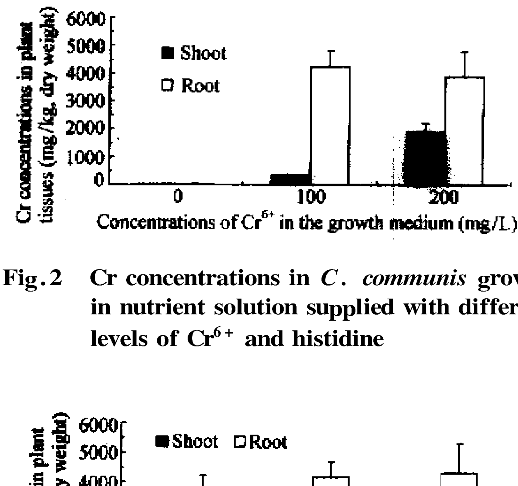

Fig.2 Cr concentrations in *C. communis* grown in nutrient solution supplied with different levels of Cr^{6+} and histidine

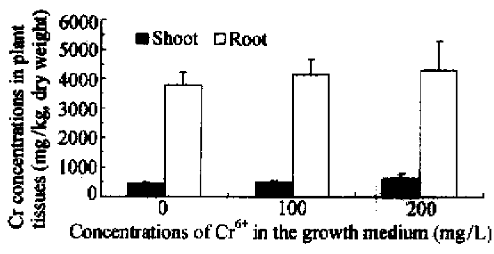

Fig.3 Cr concentrations in *C. communis* grown in nutrient solution containing Cr^{6+} 200 mg/L and different levels of histidine

that the higher histidine concentrations in the growth medium, the more Cr was accumulated in the tissues. This suggests that histidine application has positive effect on the accumulation of Cr by *C. communis* from the solution through root uptake and that much translocation of Cr to the shoot occurred when plants were supplied with histidine. Supporting evidence for this hypothesis comes from metal uptake studies when metals were supplied in chelated forms. The translocation of Cr from root to shoot was remarkably enhanced when Cr-EDTA was supplied as compared to the ionic forms of Cr (Myttenaere et al., 1974; Athalye et al., 1995; Carry et al., 1977). It should be noted that there were some differences in shoot and root accumulation of Cr by *C. communis* for the HCr treatment in experiment 1 and for the 0HisCr treatment in experiment 2 (Figs. 2 and 3). An explanation could be linked to their different growth time in the nutrient solution as suggested by the biomass production in Fig. 1 although they grew in solution containing the same level of Cr^{6+} 200 mg/L.

The addition of histidine to the hydroponic culture medium caused 16% to 46% increased chromium level in shoots, and 10 to 14 % increased chromium level in roots, although the total chromium accumulation was only marginally increased (Fig. 3). This suggests that the addition of histidine to the hydroponic solutions may provide a way to promote chromium bioaccumulation in plant shoots, a process that can be used for efficient phytoremediation of this element.

Kramer et al. (1996) investigating the role of histidine in uptake of nickel by nontolerant *A. montanum* concluded that histidine foliar spray could be more than double plant biomass at toxic concentrations of nickel and greatly alleviate the inhibitory effect of nickel on root elongation, and that supplying histidine in the root medium could increase nickel flux through the xylem of the species. In our experiment, we found that histidine application to the growth medium could enhance the uptake of Cr by *C. communis* but depress the biomass production slightly, suggesting that histidine may be involved in the mechanism of Cr tolerance and in the high rates of Cr translocation from root to shoot. The shoot/root Cr concentration ratio ranged from 0.08 to 0.48 for all tested plants grown at different concentrations of chromium and with histidine application, showing a typical pattern of excluder plants (Baker, 1981). Histidine-supplied *C. communis* attained a shoot/root Cr concentration ratio of 0.11 to 0.15, showing little variation in the increase of shoot/root ratios due to histidine application to the solution. This suggests that histidine application to the root medium could not alter the accumulation pattern of plants although it induced a higher concentration of Cr in shoots and roots of *C. communis*.

Leafy vegetables with strong ability to accumulate Fe (eg. Spinach, turnip leaves) appeared to be the most effective in translocating Cr to shoots while those with weak ability to accumulate Fe in their leaves (eg. lettuce, cabbage) are substantially less effective in translocating Cr to their leaves (Cary et al., 1977). In our study, we found that *C. communis* had substantially higher shoot/root concentration ratios than the relevant vegetables reported in literature (Zayed et al., 1998). The significantly greater shoot/root ratios for *C. communis* suggest that after Cr is absorbed by roots from nutrient solution as Cr_4^{2-}; and that Cr is comparatively highly mobile in the plant tissues. Field observation of the ecological habit of *C. communis* shows that this species is a very common species surviving on mining spoils containing high concentrations of copper, sulfur and possibly iron.

Many researches were conducted to investigate the effect of chromium on growth of agricultural crops (Chen et al., 1996). It was found that crops such as rice and wheat are very sensitive to elevated Cr concentration in the growth medium (Myttenaere et al., 1974; Chen, 1990). However, *C. communis* showed no obvious phytotoxic leaf symptoms during the experiment period although the Cr concentrations in the growth medium were very high in our experiment. This suggests that *C. communis* can tolerate Cr toxicity to some degree and may have some potential for phytoremediation. Salt et al. (1995) defined phytostabilisation as the use of certain plant species to absorb and precipitate contaminants. Species of plants best for phytostabilisation should have a good root system, tolerate high levels of the target contaminants, and retain contaminants in roots, and have minimal contaminant accumulation in aerial shoots (Miller, 1996). *C. communis* having similar features as described above may be suitable for phytostabilisation of Cr contaminated soils.

CONCLUSIONS

Hydroponic studies indicated that *C. communis* tolerated the high Cr concentrations present in the solution and also accumulated elevated concentrations of the metal in the plant tissues. The shoot and root concentrations of Cr in *C. communis* were dependent on the concentration of Cr in the growth medium and were also affected by application of histidine to the solution. Increasing Cr and histidine application to the hydroponic solution significantly increased chromium accumulation in plant tissues. However, histidine application could not alter the accumulation pattern of the plants although it induced higher concentration of Cr in shoots and roots of *C. communis*. This suggests that *C. communis* may have some potential for phytoextraction of chromium-contaminated water and in phytostabilization of Cr in mining spoils.

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