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Effect of Exogenous Organic Acids Application on Cr Uptake by *Leersia hexandra* Swartz

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A study was conducted to three different *Leersia hexandra* Swartz populations to test the effect on Cr mobilization, Cr accumulation and the growth situation of EDTA, citric acid, oxalic acid and malic acid. The results showed that EDTA had significant effect on mobilization of Cr in soil, exchangeable Cr in soil increased 80.3 % contrasting control, citric acid, oxalic acid and malic acid had passivation effect on the Cr in soil. Organic acids increased the growth rate contrasting control and citric acid increased it fastest which is 4.11 mm/day; EDTA promoted the Cr uptake significantly. The Cr content in the roots was 3 times higher than that of control. The migration coefficient increased by 24 % compared to control, citric acids had no influence on mobilization of Cr and accumulation, however, oxalic acid and malic acid retrained the mobilization of Cr, decreased the accumulation of *Leersia hexandra* Swartz slightly.

Key Words: Chromium, Organic acids, EDTA, *Leersia hexandra* Swartz.

INTRODUCTION

The use of phytoremediation on heavy metal contaminated soil and water had been widely studied and applied in recent years. Many scholars studied further the effect of exogenous organic acids on the rate of hyperaccumulator distilling heavy metal. Some studies showed that EDTA added to the soil might participate the transportation and accumulation of heavy metal and promoted the hyperaccumulator of heavy metal on plants[1-5]. However, some studies reported that adding EDTA would restrain plants from accumulating heavy metal[6], Liphadzi's study showed that the enriched effects on different heavy metal of EDTA with different concentrations were not the same[7]. The effects of other organic acids on mobilization of heavy metal in soil and the accumulation of heavy metal for plants differed from the categories of organic acids or heavy metal[8]. Citric acid, oxalic acid and malic acid influenced the Cr uptake by *Leersia hexandra* Swartz to some extent[9,10]. Thus, the regulation mechanism of organic acids for the plants absorbing heavy metals need to be further studied. The experiment studied the effect of organic acids on Cr uptake by *Leersia hexandra* Swartz in three fields, provided theoretical basis for phytoremediation of Cr wastesoil.

EXPERIMENTAL

Three *Leersia hexandra* populations were collected from Lipu County, Yanshan District and Taohuajiang in Guilin. Test soil was paddy soil and its N concertration is 1700-1900 mg kg^{-1}; P 470-600 mg kg^{-1}; K 4000-5200 mg kg^{-1}; Hg 0.33 mg kg^{-1}; As 7.48 mg kg^{-1}; Pb19.0 mg kg^{-1}; Cd 0.24 mg kg^{-1} and its pH is 7.22.

Design and methods: The soil was air dried, passing sieve with 20 mesh, then be added into the Cr^{3+}($CrCl_3$) solution with concentration of 400 mg kg^{-1}, set aside after balancing for 2 weeks. In the experiment,organic acid was set into 4 concentration gradients, that is, the control 0, 4 and 8 mmol L^{-1}. Four replicates in each treatment. After 2 weeks, gained the *Leersia hexandra* Swartz, disjoined it by roots, stems and leaves.

Determination of Cr in plant samples: Placed the fresh samples into an oven at 105 ºC for 0.5 h, then cut to small pieces and dried at 80 ºC for 24 h. The dried plant samples were ground and 1 g of the powder was taken and carefully digested with 5:1 (v/v) mixture of concentrated HNO_3 and $HClO_4$. The solution obtained after digestion was analyzed for heavy metal content with flame atomic accumulation spectrophotometry (GBC, Avanta).

Determination of exchangeable Cr in soil: To weight 5 g fresh soil sample in 100 mol plastic centrifuge tube, add 1 mol L^{-1} of $MgCl_2$ (pH 7.0) solution in a mass ratio of soil and water of 8:1, flask at 25 ºC with shaking for 2 h, centrifuged for 15 min, determined the Cr content by the Hitachi Z-8000 atomic accumulation spectrophotometer.

RESULTS AND DISCUSSION

Effect on the growth and development of *Leersia hexandra* Swartz of different organic acids: As shown in Table-1, after the organic acids were utilized into the soil, the growth rate increased compared to control, the growth rate increased faster when treated by citric acids. However, the difference among each other was not significant ($p = 0.086 > 0.05$). This might be that adding organic acids relieved the toxicity of heavy metal in some content and accelerated the growth of crop. It is also reported that addition of citric acid could decrease toxicity of Cd to the wheat and rice seeds[11]. Through solution culture experiments, it was found that citric acid and oxalic acid could decrease toxicity of Cd, increase the dry weight and fresh weight of wheat[12]. Oxalic acid could increase the absorptive of soil to Cr and decrease its toxicity[13]. The dry weight of *Leersia hexandra* Swartz was affected insignificantly by organic acids ($p = 0.323 > 0.05$). Although the root length and maximal leave length increased, their differences is not significant ($p = 0.238, 0.327 > 0.05$). It showed that organic acids take on some detoxification, because *Leersia hexandra* Swartz didn't behave obvious poisoned symptom on heavy metal, but the effect on biomass of organic acids was not significant.

Effect on exchangeable Cr of different organic acids: The effect of different organic acids on exchangeable Cr content was significantly different. As shown in Fig. 1, adding citric acid, oxalic acid and malic acid decreased the exchangeable Cr content slightly, but the effect was not significant. ($p = 1.184 > 0.01$). However, treatment with EDTA increased the exchangeable Cr content significantly,increased 80.3 % compared with control. This showed that citric acid, oxalic acid and malic acid reduced the activities of Cr in soil, promoted the dissolving effect of Cr.

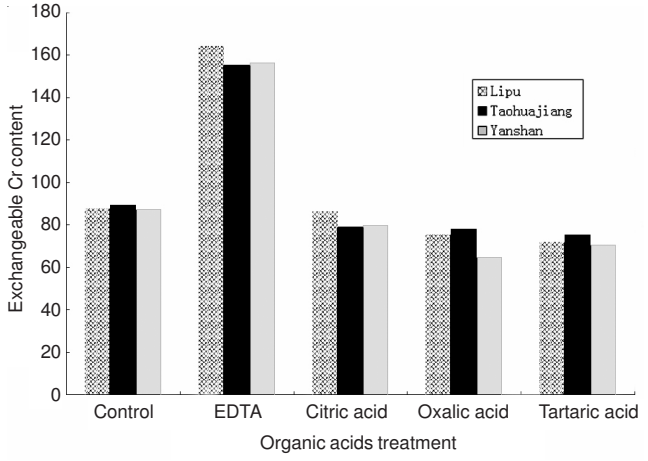

Fig. 1. Effect of different organic acids on exchangeable Cr in soil

Effect on root accumulation of Cr by *Leersia hexandra* Swartz of different acids: Effect of different exogenous acids on root accumulation of Cr by *Leersia hexandra* Swartz are shown in Fig. 2. The addition of EDTA increased the root accumulation of Cr by *Leersia hexandra* Swartz significantly, the Cr content of root was 3671 mg kg^{-1}, which was 3 times of control, differing from addition of other organic acids significantly ($p = 0.02 < 0.05$), which was in good-agreement with the dissolving effect of Cr by organic acids. Treatment with other organic acids showed little effect on Cr content, no significant difference was found comparing with control.

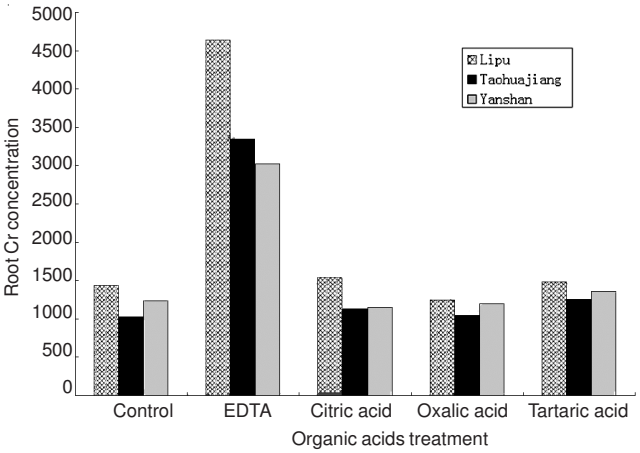

Fig. 2. Effect on root Cr concentration in *Leersia hexandra* Swart of different organic acids

Effect on the transfer of Cr of different organic acids: Effect on the transfer of Cr of different exogenous organic acids are shown in Fig. 3. The effect of phytoremediation depended on the uptake of heavy metals by plants and more important, the transfer capacity of heavy metals from root to its overground[14]. The transfer capacity of heavy metals in plants is expressed by transfer coefficient that is the ratio of Cr content in overground parts and in roots. As shown in Fig. 3, adding EDTA increased the transfer capacity of Cr to overground parts,transfer coefficient is 1.44 compare with 1.16 of control, increasing by 24 %; citric acid played a certain role in migration of Cr, but the effect was not more obvious than that of EDTA; oxalic acid and malic acid even decreased the transfer capacity of Cr to overground parts slightly, transfer coefficient reduced by 0.11 and 0.08, respectively compared with that of the control, the results showed that oxalic acid and malic acid had certain inhibition effect on transfer capacity of Cr. This might be related to the lower solubility of the complex formed by oxalic acid, malic acid and Cr.

TABLE-1
EFFECT ON GROWTH OF *Leersia hexandra* SWART OF DIFFERENT ORGANIC ACIDS

Treatment	Growth rate (mm/d)	Biomass (g pot^{-1} DW)		Maximal leave length (mm/d)	Root length (mm)
		Above ground	Under ground		
Control	2.68 ± 0.02b	7.56 ± 0.43c	3.22 ± 0.13bc	0.16 ± 0.03b	145.0 ± 31.4c
EDTA	3.25 ± 0.07ab	15.07 ± 0.28ab	5.27 ± 0.18ab	0.33 ± 0.11b	212.5 ± 25.8b
Citric acid	4.11 ± 0.11a	21.33 ± 0.32ab	7.28 ± 0.33ab	0.45 ± 0.08ab	350.7 ± 39.3a
Oxalic acid	3.50 ± 0.05a	10.43 ± 0.25b	4.20 ± 0.03b	0.24 ± 0.04b	258.8 ± 11.8b
Tartaric acid	3.13 ± 0.06ab	10.30 ± 0.19b	4.26 ± 0.19b	0.20 ± 0.09b	202.5 ± 31.9b

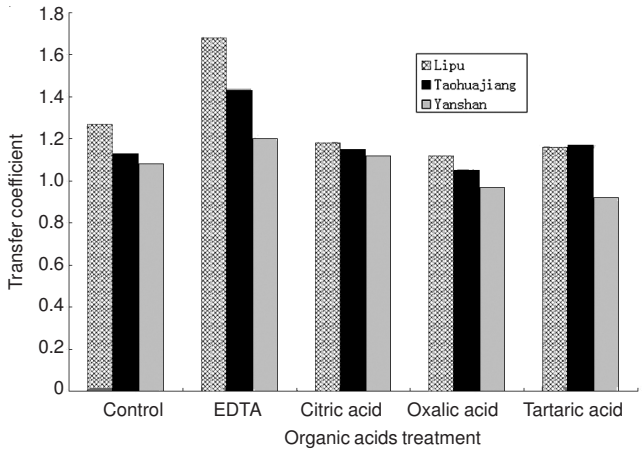

Fig. 3. Effect of different organic acids on transfer coefficient

Conclusion

Making use of organic acids to enhance the activity of heavy metal could promote the uptake of metals by plants, which is one of research directions of phytoremediation. Effect of organic acids on the uptake of metals by plants was not only depended on the kinds and characteristics of organic acids, but also on environmental conditions and the plant species. As for the Cr, addition of EDTA could enhance significantly the activity of Cr, not only increased the uptake of Cr by *Leersia hexandra* Swartz, but also promoted its transfer to overground, which was highly favourable to the success of phytoremediation. Blaylock's study showed that the effect of EDTA on promoting the activation and accumulation was especially significant, in the soil polluted by 600 mg kg^{-1} Pb, adding 10 mmol kg^{-1} EDTA, the Pb content of *Leersia hexandra* Swartz could get to 1.6 % in the overground[5]. Addition of EDTA into the soil, Pb could be accumulated and transported by plants in the form of Pb-EDTA in the soil[15]. The effect of citric acid, oxalic acid and malic acid on Cr was distinctly different from EDTA[16], addition of citric acid, oxalic acid and malic acid rather decreased the content of exchangeable Cr in soil than promoted the activation of Cr, it might be the binding ability of citric acid, oxalic acid and malic acid to Cr was weaker, the formed complex was not stable and wonderfully compliant about being broken down, which increased the carbonate content in soils, promoted the absorptive capacity of soil to Cr. However, this did not affect the uptake of Cr by *Leersia hexandra* Swartz in the overground. This might be due to the effect of citric acid, oxalic acid and malic acid on Cr didn't get to the threshold that affect the plants accumulation, the conclusion was consistent with the studies about the effect of organic acids on the uptake of Cu and Cd by *Leersia hexandra* Swartz and Indian mustard[17,18].

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