Assessing the Phytotoxicity of Cr(III) and Cr(VI) in Cr(VI)-Spiked Soils by Using XANES and Resin Extraction Methods

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Abstract
Simultaneously determining the two species, Cr (III) and Cr (VI), under different organic matter contents and pH levels would be required for assessing Cr phytotoxicity in contaminated soils. Two acid soils (Neipu and Pinchen) were adjusted within three pH ranges (4-5, 5-6, and 6-7) and then treated with four Cr (VI)-spiked levels (0, 500, 1000, and 1500 mg/kg). The extent of Cr (VI) reduction was determined by using X-ray absorption near edge structure spectroscopy (XANES). Two selective exchange resins (Chelex 100 and DOWEX M4195) were used to extract Cr (III) and Cr(VI) separately from soil samples. And, wheat seedling experiment was conducted for illustration of Cr phytotoxicity. The results showed that in Neipu soil, Cr (VI) was mostly reduced to Cr (III) by the larger amount of organic matter. Chelex 100 extractable Cr (III) was decreased with raising pH and then the injury of seedlings was significantly reduced. Nevertheless, according to the results of XANES spectra, in Pinchen soil, Cr (VI) was not completely reduced to Cr (III). Dowex M4195 extractable Cr (VI) was increased with raising pH. And, injury of seedlings was increased with raising the Cr (VI)-added levels. Thus, Cr phytotoxicity in the injury of seedlings was dominated by Cr(VI) in Pinchen soil.

Key Words
Chelex 100, Dowex M4195, reduction.

Introduction
Chromium(VI) is carcinogenic due to its high mobility and toxicity, and exists in the environment as $Cr_2O_7^{2-}$ or CrO_4^{2-}; Cr(III) is less soluble and is considered to be less toxic than Cr(VI). Organic matter in soil acts an predominant electron donor which contributed to the reduction of Cr (VI) to Cr (III). The reaction is pH dependent and is enhanced with decreasing pH (Adriano 1986). It has been evidenced that the solubility of Cr(III) increases with decreasing pH, and the phytotoxicity of Cr(III) under acid condition is rising due to its high solubility (Han *et al*. 2004). It is assumed that the phytotoxicity of Cr in Cr (VI)-contaminated soil with various soil pH levels and organic matter content could be attributable to both Cr(VI) and Cr(III) or either of them. Therefore, the phytotoxicity of Cr(VI) and Cr(III) in Cr(VI) - contaminated soil need to be clarified. The XANES spectroscopy provide nondestructive measurement of oxidation states of chromium on the soil surface and is a useful tool to estimate the degree of Cr(VI) reduction (Bang and Hesterberg 2004; Lee *et al*. 2006). In this study, Dowex M4195 (Yu *et al*. 2004) and Chelex 100 resins (Chen *et al*. 2008), were used to assess the phytotoxicity of Cr(VI) and Cr(III) in Cr(VI)-contaminated soil with various soil pH levels and organic matter content condition. In addition, the wheat seedling growth experiment was used to determine the phytotoxicity effect of soil Cr.

Methods
Two major agricultural acid soils, Pinchen (pH = 4.3; organic matter = 27.3 g/kg; Fe_d (Dithionite-citrate-bicarbonate extractable iron) content = 27.7 g/kg) and Neipu (pH = 4.1, organic matter = 93.7 g/kg; Fe_d content = 13.2 g/kg), from Taiwan were used. Soil pH of Neipu and Pinchen soils were adjusted within three ranges for 4-5, 5-6 and 6-7 by different rate of $CaCO_3$ addition. Limed soils were mixed thoroughly and incubated at room temperature for 1 month. Chromium(VI) ($K_2Cr_2O_7$) were then spiked into soils to reach four levels of Cr(VI), and then underwent three wetting-drying cycles for three weeks at room temperature. The soil samples were ground and sieved through a 425 µm sieve for phytotoxicity experiment, a 177 µm sieve for resin Cr extraction and sequential extraction, and a 63 µm sieve for XANES analysis.

The DOWEX M4195 resin was saturated with 500 mg/L $CuCl_2$ and converter into Cu-saturated form. The

Cu-saturated resin was retained in 425 μm polypropylene (PP) bags. The Chelex 100 resin was saturated by 2 M $CaCl_2$ and converted into Ca-saturated form. Then, the Ca-saturated resin was retained in 177 μm PP bags. Ten gram soil samples were placed into 300 mL flasks, and then 100 mL distilled water was added. One gram of Chelex 100 resin and 2 g of DOWEX M4195 resin were added into separate flasks and shaken at 25 ℃ for 24 hrs separately. After Cr extracted, the Chelex 100 resin and the DOWEX M4195 resin were washed with distilled water and placed into 100 mL of 2 M H_2SO_4 and 100 mL of 10 % NaCl respectively to desorb the Cr adsorbed on resins. The Cr concentration in solution was determined by ICP-AES (Inductively Coupled Plasma Atomic Emission Spectroscopy, Perkin Elmer, Optima 2000DV).

The XANES spectra were collected from National Synchrotron Radiation Research Center of Taiwan. The X-ray absorption Cr K-edge (5989 eV) spectra were obtained using a wiggler beam line, BL-17C1. The modified Neübauer method (Yu *et al*. 2004) was used to investigate the phytotoxicity of soil Cr to wheat seedlings (*Triticum Vulgare*, variety Taichuang select No. 34). The experiment was carried out for 25 days in the Phytotron of the National Taiwan University. A modified sequential fractionation scheme of Tessier *et al*. (1979) was used to clarify the distribution of Cr (III) reduced from Cr (VI) in Neipu soil.

Results

Although the highest pH level (pH = 6.4) was not effective in reducing Cr(VI) in Neipu soil, there was no detectable Cr(VI) peak in all rates of $CaCO_3$ addition (Figure 1 (a)). Thus, the Cr(VI) reduction was not regardless of soil pH. In the case of Pinchen soil, the degree of Cr(VI) reduction was relate to soil pH (Figure 1 (b)). The intensity of the characteristic Cr(VI) peaks in XANES spectrum increased with increasing soil pH.

The Dowex M4195 resin extractable Cr was not detected in Neipu soil in all Cr treatments at each pH level (Table 1), whereas generally increased with increasing Cr(VI) addition and pH levels in Pinchen soil. It is suggested that Cr(VI) was most probably reduced into Cr(III) by larger amount of organic matter in Neipu soil. Due to the organic matter content in Pinchen soil is lower, soil pH became the main factor that appreciably affected Cr(VI) reduction. Additionally, the greater amount of Chelex 100 extractable Cr was detected in Pinchen soil than that in Neipu soil, which increase with Cr(VI) addition and decreased with increasing soil pH (Table 1). It is indicated that the reduction of Cr(VI) increased soil pH due to the proton consumption reaction (Bolan and Thiagarajan 2001), and reduced the solubility of Cr(III) reduced from Cr(VI) in Neipu soil than that in Pinchen soil. Moverover, the reduced product Cr(III) was mainly associated with the organic bound and Fe-Mn oxide bound fractions in Neipu soil.

In Cr (VI)-spiked Neipu soil, plants were not injured in all Cr(VI) treatments for each pH level (Figure 2), whereas the plant height of wheat seedlings increased with increasing Cr(VI) addition due to the increase in pH facilitated the plan growth. In the case of Pinchen soil, the plant height of wheat seedlings approximately decreased with increasing Cr(VI) concentration and soil pH. It is suggested that the injury of plants could be attributed to both Cr(VI) and the Cr(III) reduced from Cr(VI). However, the amount of soil Chelex 100 extractable Cr was quite low and the larger amount of spiked Cr (VI) was not reduced to Cr(III). Thus, Cr(VI) was supposed to be responsible for phytotoxicity.

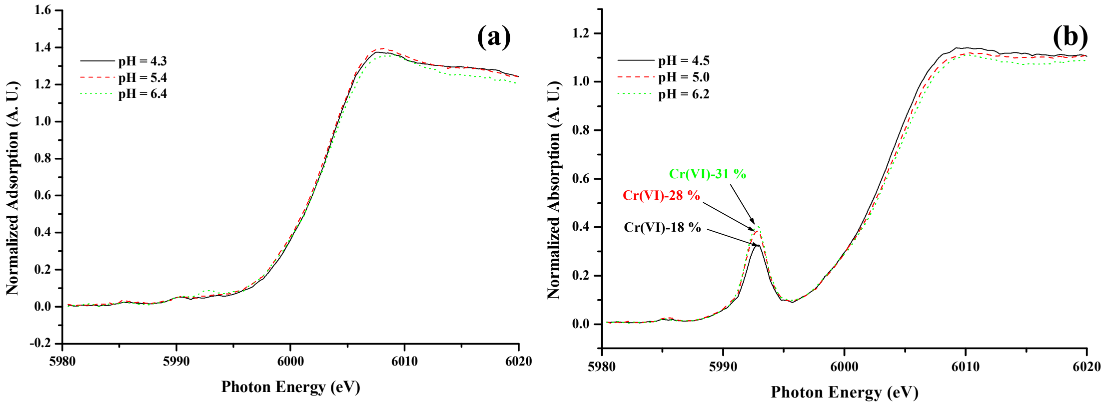

Figure 1. The XANES spectra of Cr(VI)-spiked soil (1500 mg Cr(VI)/kg soil) at different pH. (a) Neipu; (b) Pinchen soil.

Table 1. The amounts of Chelex 100 and Dowex M4195 resin extractable Cr in Cr(VI)-spiked soils with different soil pH.

Soils	Soil Cr(VI) (mg/ kg)	Resin extractable Cr (mg /kg)					
		pH =4.3		pH =5.4		pH = 6.4	
		Chelex 100	Dowex M4195	Chelex 100	Dowex M4195	Chelex 100	Dowex M4195
Neipu soil	Control	ND^{dAB}	ND	ND^{d}	ND	ND^{c}	ND
	500	$5.1^{b}\pm0.9$	ND	$4.0^{b}\pm0.4$	ND	$2.5^{b}\pm0.7$	ND
	1000	$8.4^{a}\pm1.1$	ND	$8.2^{a}\pm1.2$	ND	$6.0^{a}\pm0.3$	ND
	1500	$8.6^{a}\pm2.3$	ND	$8.3^{a}\pm1.7$	ND	$6.1^{a}\pm1.8$	ND
		pH = 4.5		pH = 5.0		pH = 6.2	
		Chelex 100	Dowex M4195	Chelex 100	Dowex M4195	Chelex 100	Dowex M4195
Pinchen soil	Control	ND^{f}	ND^{e}	ND^{d}	ND^{e}	ND^{d}	ND^{d}
	500	$3.7^{c}\pm0.1$	$9.2^{c}\pm0.6$	$4.1^{c}\pm0.0$	$17.8^{c}\pm0.7$	$1.5^{c}\pm0.1$	$13.1^{c}\pm0.1$
	1000	$11.6^{b}\pm0.4$	$40.7^{b}\pm0.0$	$12.7^{b}\pm2.3$	$62.0^{b}\pm1.7$	$9.2^{b}\pm0.0$	$65.2^{b}\pm0.0$
	1500	$20.9^{a}\pm0.1$	$96.3^{a}\pm1.8$	$22.0^{a}\pm0.5$	$130.5^{a}\pm3.0$	$24.0^{a}\pm1.1$	$223.8^{a}\pm7.9$

[A]Values followed by the same letter, within the same column and type of soil, are not significantly different (Duncan Multiple Range Test, p =0.05).
[B]Detection limit (≤ 0.05 mg/kg)

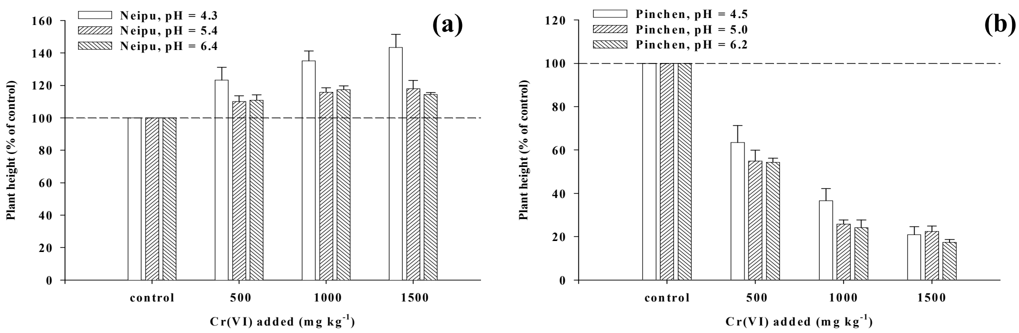

Figure 2. The plant height of wheat seedlings in Cr(VI)-spiked soils with different soil pH. (a) Neipu soil; (b) Pinchen soil.

Conclusion

Cr(VI) added into Neipu soil was mostly reduced to Cr(III) by the large amount of organic matter, and the Cr(VI) reduction was regardless of soil pH. The decrease of phytotoxicity of Cr(III) reduced from Cr(VI) due to the solubility of Cr(III) decreased while proton consumption reaction causing soil pH increasing and binding with organic matter and the formation of precipitation. In Pinchen soil, DOWEX M4195 resin extractable Cr increased with increasing pH, and the greater amount of Chelex 100 extractable Cr was detected than that in Neipu soil. The injury of wheat seedlings can be attributed to both Cr(VI) and Cr(III). However, the large amounts of available Cr(VI) was supposed to be dominant for phytotoxicity.

Acknowledgement

This research was sponsored by the National Science Council, Taiwan, Republic of China under grant No. NSC 95-2313-B-002-076-MY3.

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