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# Effect of EDTA on Copper and Cadmium Absorption in *Pharbitis nil* and *Triticum aestivum*

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The addition of 30 μM EDTA to the medium inhibited the absorption of copper, but not that of cadmium in *Pharbitis nil* and *Triticum aestivum*, as was the case for *Lemna*. However, ammonium added to the medium did not decrease the absorption of copper or cadmium in both plants, unlike the case for *Lemna*.

Key words: Ammonium -- Cadmium -- Copper -- EDTA -- *Pharbitis nil* -- *Triticum aestivum*

Chelating agents, such as EDTA, have been said to promote the absorption of iron and copper in plants. In the previous paper (Tanaka et al. 1982), however, we reported that the addition of EDTA to the medium inhibited the absorption of copper in *Lemna paucicostata* 6746 and *Lemna gibba* G3. Since Hillman (1961) indicated that root of *Lemna* seems to be degenerated to only a trace organ, the absorption mechanism of heavy metals in *Lemna* might differ from those in other plants. In the present paper, therefore, we examined the effect of EDTA on the absorption of copper and cadmium by *Pharbitis nil* and *Triticum aestivum*.

Seeds of *Pharbitis nil*, Violet, were treated with concentrated sulfuric acid for 30 min, washed in running water overnight and grown in moist vermiculite. Six days later, when the cotyledons unfolded, they were transplanted to test tubes (15x105 mm) containing 10 ml of experimental medium. Seeds of *Triticum aestivum*, Asakaze, were soaked in running water overnight and placed on moist filter paper. Four-day-old seedlings with 20-30 mm coleoptiles were planted in test tubes (15x105 mm) containing 5 ml of experimental

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medium after the removal of endosperms. Modified Hoagland medium ( M medium, Hillman 1961 ) with an initial pH of 4.1 was used as the basic medium.

Both plants were grown for 3 days on the experimental medium under continuous daylight fluorescent light (6000 lux) at 25±1°C, then washed successively with distilled water, 1% EDTA solution at pH 5.0 and distilled water for 1 min each. The methods to determine the amount of copper and cadmium accumulated in plant bodies were similar to those described previously (Tanaka et al. 1982, Nasu et al. 1983). The concentration of copper and cadmium was expressed on a fresh weight basis. Three experimental cultures were used for each treatment, and the experiments were repeated at least twice.

When *Pharbitis nil* and *Triticum aestivum* were grown on M medium containing various concentrations of $CuSO_4$ without EDTA, the copper concentration in the plants rose with the increase in the copper concentration in the medium (Fig.1). The copper concentration of *Triticum aestivum* was higher than that of *Pharbitis nil*. Fresh weight after 3-day culture was about 1.15 and 1.8

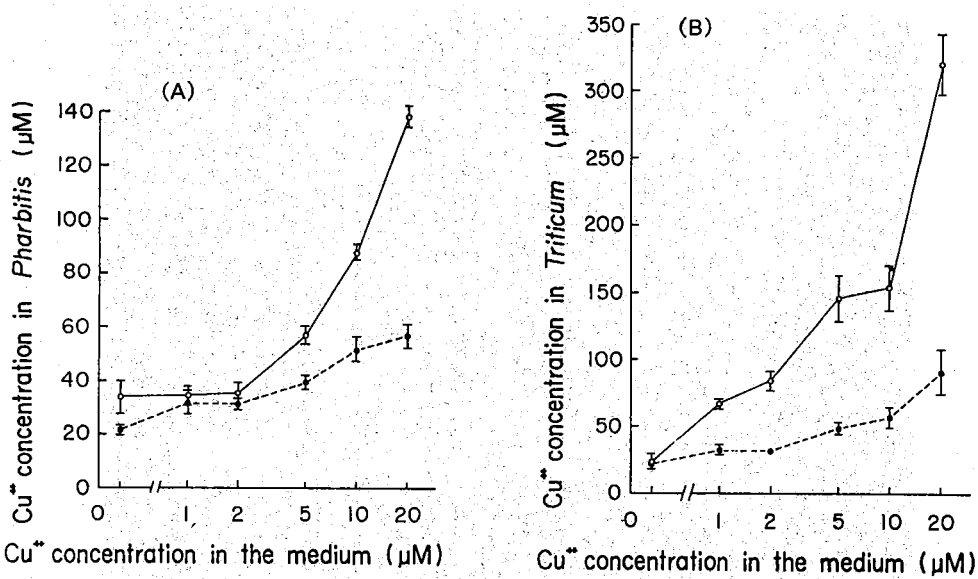


Fig. 1 Copper concentration in *Pharbitis nil* (A) and *Triticum aestivum* (B) cultured on M medium containing various concentrations of $CuSO_4$ with or without 30 μM EDTA added. Plants were grown under continuous light of 6000 lux at 25±1°C for 3 days. Results are presented as the mean ± standard error.

O———O : without EDTA

●- - - - - - ● : with 30 μM EDTA

times greater than that of start of treatment in *Pharbitis nil* and *Triticum aestivum*, respectively. No significant change in fresh weight was seen in both plants even on the medium containing 20 μM $CuSO_4$, but *Pharbitis nil* grown on the medium containing more than 10 μM copper could not develop any adventitious roots at the base of the hypocotyl (Fig.2).

The absorption of copper by these two plants was clearly reduced by the addition of 30 μM EDTA to the medium (Fig.1). The formation of adventitious roots in *Pharbitis nil* was completely recovered by simultaneous addition of EDTA to the medium (Fig.2).

These results indicate that 30 μM EDTA added to the medium suppresses the absorption of copper in *Pharbitis nil* and *Triticum aestivum* as was the case for *Lemna*.

The cadmium absorption in *Lemna* is not inhibited by the addition of 30 μM EDTA to the medium (Nasu et al. 1983). Therefore, we next examined the effect of EDTA on cadmium absorption in *Pharbitis nil* and *Triticum aestivum*.

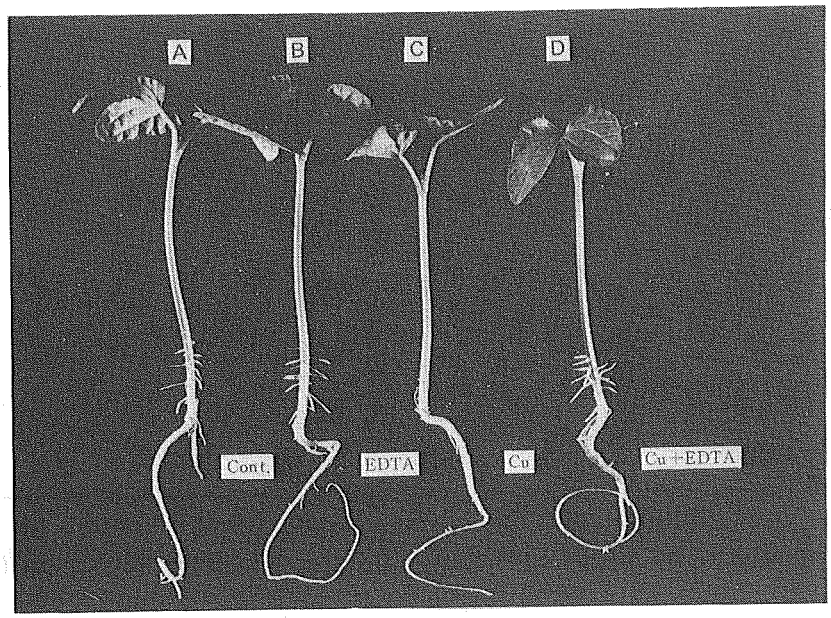


Fig. 2 Effects of copper and EDTA on the formation of adventitious root formation in *Pharbitis nil*. For culture conditions, see the legend to Fig.1.

A : grown on the medium with neither copper nor EDTA

B : grown on the medium with 30 μM EDTA

C : grown on the medium with 10 μM $CuSO_4$

D : grown on the medium with both 10 μM $CuSO_4$ and 30 μM EDTA

These plants were cultured for 3 days on M medium containing various concentrations of $CdCl_2$ with or without 30 μM EDTA. The cadmium concentration in both plants rose with the increase in the cadmium concentration of the medium, irrespective of the presence or absence of 30 μM EDTA in the medium (Fig.3).

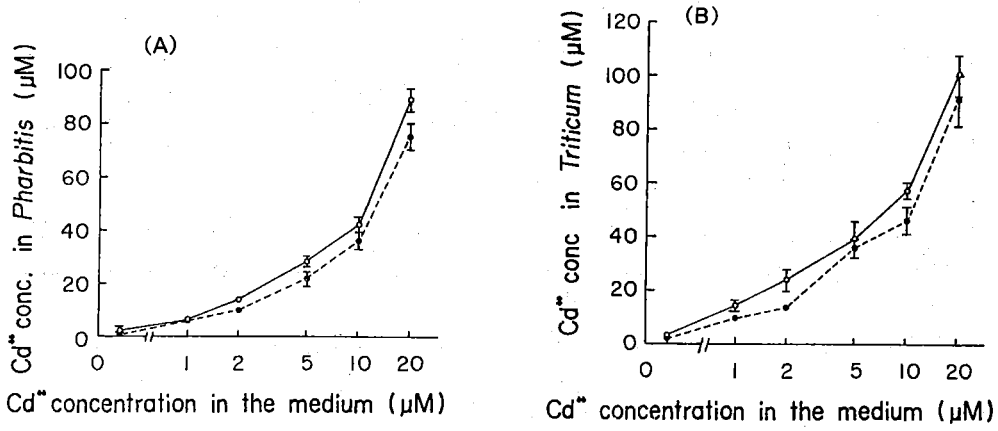


Fig. 3 Cadmium concentration in *Pharbitis nil* (A) and *Triticum aestivum* (B) cultured on M medium containing various concentrations of $CdCl_2$ with or without 30 μM EDTA added. For culture conditions, see the legend to Fig.1.

○———○ : without EDTA

●- - - - - ● : with 30 μM EDTA

That is, 30 μM EDTA did not prevent the absorption of cadmium in *Pharbitis nil* and *Triticum aestivum*.

In *Lemna*, ammonium ion added to the medium greatly suppresses copper absorption (Tanaka et al. 1982) and slightly suppresses cadmium absorption (Nasu et al. 1983). In the next experiment, *Pharbitis nil* and *Triticum aestivum* were grown on M medium containing various concentrations of ammonium with 10 μM $CuSO_4$ or 10 μM $CdCl_2$ under continuous illumination for 3 days. Copper and cadmium contents of both plants at the end of the culture period are shown in Fig.4. The addition of ammonium to the medium had no significant effect on the copper and cadmium accumulation in either plant, unlike the case for *Lemna*. What causes such a difference between *Lemna* and *Pharbitis nil* or *Triticum aestivum* is unknown, but the action of EDTA on copper and cadmium absorption seems to be similar in these three plants.

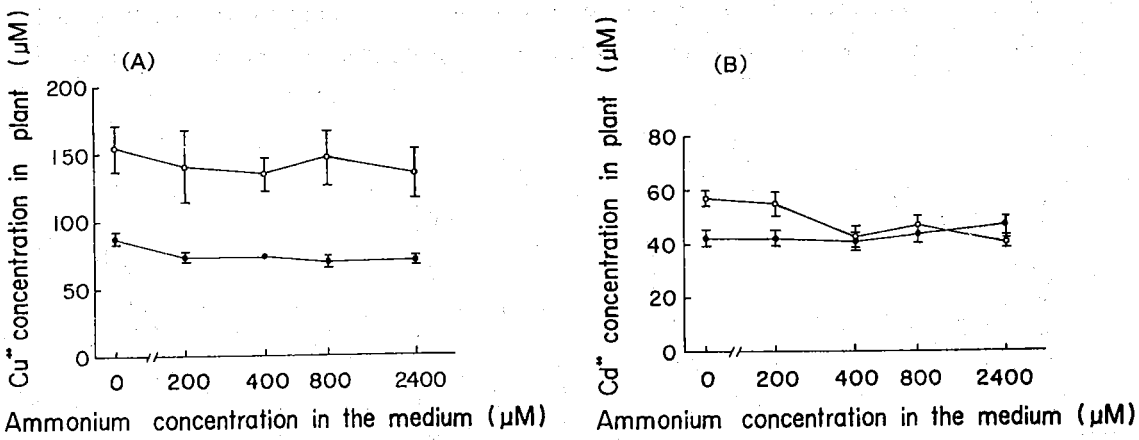


Fig. 4 The concentration of copper (A) and cadmium (B) in *Pharbitis nil* and *Triticum aestivum* cultured on M medium containing various concentrations of $NH_4Cl$ together with 10 μM $CuSO_4$ (A) or 10 μM $CdCl_2$ (B). For the culture conditions, see the legend to Fig.1.

○——○ : *Triticum aestivum*

●——● : *Pharbitis nil*

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