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Biosorption of trace metals from aqueous multi-metal solutions by green microalgae

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Abstract Two strains of green microalgae *C. reinhardti* and *C. pyrenoidosa* were examined for their biosorption of Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} from aqueous multi-metal solutions. A wide range of biosorption capacities can be observed due to different strains of microalgae and different species of trace metals. This characteristic was ascribed to the distinct components and structures of algal cell walls and the different physicochemical properties of trace metals, such as atomic weight and ion density. *C. pyrenoidosa* showed higher uptake capacities than *C. reinhardti* and both of them had a preference for the uptake of cadmium over others in the trace metal solution, suggesting they can be a good biomaterial for biosorption of cadmium. Live microalgal cells displayed a more complex sorption process than dead microalgal cells because of cell assimilation.

Key words biosorption; trace metal; microalga

1 Introduction

Since the industrial revolution, human activities have strongly changed the primordial distributions of trace metals in the Earth's surface layer, thus imposing a potential threat on the environment and human health. Algae are sensitive to environmental change and their physiologic functions such as photosynthesis and main nutrients assimilation can be influenced by the variation of trace metals (Danilov and Ekelund, 2001; Devriese et al., 2001; Wang Baoli et al., 2005). Therefore, algae have been proved to be an excellent biomonitor for trace metal pollution (Falasco et al., 2009). Moreover, algae can avidly accumulate trace metals (Morel and Price, 2003) and nowadays become an interesting biosorbent due to their high adsorption capacities and low cost (Klimmek et al., 2001; Priyadarshani et al., 2011).

Biosorption has been defined as the property of certain biomolecules (or types of biomass) to bind and concentrate selected ions or other molecules from aqueous solutions (Volesky, 2007). As opposed to a much more complex phenomenon of bioaccumulation based on active metabolic transport, biosorption by dead biomass (or by some molecules and/or their active groups) is passive and based mainly on the "affinity" between (bio-)sorbent and sorbate (Volesky, 2007). While the biosorption of trace metals has become a hot topic, few studies have been conducted on algal biosorption and most studies have focused on a single metal in preference to multi-component systems at present (Febrianto et al., 2009; Romera et al., 2006; Volesky, 2007). In this study, we investigated biosorption of Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} from aqueous multi-metal solutions by live and dead green algae *C. reinhardti* and *C. pyrenoidosa*. Our aim is to better understand the species-specific characteristics of algae and trace metals and the mechanism controlling the biosorption in aqueous multi-metal solutions.

2 Methods

2.1 Algal axenic culture

C. reinhardti and *C. pyrenoidosa* were obtained from Institute of Hydrobiology, CAS and axenically cultured in the artificial freshwater medium SE (http://algae.ihb.ac.cn). The concentrations of Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} in SE medium

were 1×10^{-2}, 2×10^{-2}, 0, 0, 3×10^{-4}, 7×10^{-4}, and 0 mmol·L^{-1}, respectively. *C. reinhardtii* and *C. pyrenoidosa* were cultured in 150 mL flasks in an incubation chamber at 25.0±1.0℃, under a 16:8 h light:dark regime with 4000 LX illumination. The contents of chlorophyll *a* (Q_{chla}) were used to show microalgal biomass. Chlorophyll *a* was extracted with 90% ethanol and measured by spectrophotometry (Lorenzen, 1967).

2.2 Biosorption of trace metals by algae

All chemicals used in this study were of A.R. grade. Trace metal stock solutions consisted of distilled water and $MnSO_4·H_2O$, $FeSO_4·7H_2O$, $CoCl_2·6H_2O$, Ni $(NO_3)_2·6H_2O$, $CuSO_4$, $ZnSO_4·7H_2O$, and $CdCl_2·2.5H_2O$, respectively. The concentration of each kind of trace metals in each stock solution was 10^{-2} mol·L^{-1}.

By centrifugation at 1600 g for 10 min, *C. reinhardtii* and *C. pyrenoidosa* were obtained. Interaction among metal ions and the variation of pH value can affect biosorption of metal ions. So, in order to eliminate the effects of other metals on biosorption, only distilled water was added in the centrifuged algae (Puranik and Paknikar, 1999). The dead algal cells were obtained by boiling the reactive solution for about 1 min. Results of observation under light microscope showed that algal cells were not destroyed in these boiled reactive solutions. The conditional experiments indicated that the biosorption of trace metals by these microalgae reached balance in 10 min in this study, and this is consistent with Slaveykova and Wilkinson (2002).

By adding trace metal stock solution in reactive solution, *C. reinhardtii* and *C. pyrenoidosa* were treated under Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} exposure conditions, for live cells at 0.01, 0.02, 0.03, 0.05, 0.07, and 0.1 mmol·L^{-1} (called C_0) and for dead cells at 0.01, 0.02, 0.05, 0.07, and 0.1 mmol·L^{-1}, respectively. After 10 min, reactive solution was immediately filtered with a cellulose acetate membrane filter (0.45 μm pore size, Millipore), and the filtrate was used to determine the trace metal concentrations (called C_e) by atomic absorption spectrophotometry (PE-5100-PC). The quantity of biosorption on trace metals by the microalgae was calculated according to the following equation: $Q=(C_0-C_e)/Q_{chla}$, where Q is the quantity of biosorption. Treatments were conducted in an incubation chamber at 25.0±1.0℃, under a 16:8 h light:dark regime with 4000 LX illumination and reciprocative shaking (90 times/min). Each treatment consisted of three replicates and the volume of each replicate was 20 mL.

3 Results

Freundlich adsorption isotherm was used to describe adsorption equilibrium of trace metals by green microalgae *C. reinhardtii* and *C. pyrenoidosa* in this study. It is an empirical equation expressed by the following formula: $Q=K\cdot C_e^n$. The results indicated that the quantity of biosorption of each kind of trace metals except Ni increased with increasing trace metal concentrations, and these trace metals except Ni showed a significant relationship between Q and C_e (Figs. 1 and 2).

C. reinhardtii and *C. pyrenoidosa* showed different abilities of sorption for each kind of trace metals. Generally, *C. pyrenoidosa* adsorbed more trace metals than *C. reinhardtii* did (i.e., $y_P/y_R>1$; Table 1). For example, *C. pyrenoidosa* had twice more Q for Cd by live cells than *C. reinhardtii*. Q of *C. pyrenoidosa* was found less than that of *C. reinhardtii* at lower trace metal concentrations only for biosorption of Zn^{2+} by live cells and for biosorption of Co^{2+}, Fe^{2+} and Mn^{2+} by dead cells (Table 1).

Live algal cells presented different adsorbabilities from dead ones. In the case of Fe^{2+}, live cells of both microalgae adsorbed much more Fe^{2+} than dead cells did. As for Zn^{2+}, Q of dead cells showed a sharp increase than that of live cells with increasing Zn^{2+} concentrations. However, Q of Cu^{2+} absorbed by live *C. reinhardtii* exhibited a similar change to that of dead *C. reinhardtii* with the variation of Cu^{2+} concentrations.

Table 1 Calculation of the Freundlich regression equation for biosorption of each kind of trace metals by live and dead microalgae *C. pyrenoidosa* and *C. reinhardtii*, respectively

	$f(x)_{live}=y_P/y_R$	$f(x)_{dead}=y_P/y_R$	$f(x)_P=y_{live}/y_{dead}$	$f(x)_R=y_{live}/y_{dead}$
Cd^{2+}	$1.97x^{-0.02}$ (2.05－2.13)[a]	$9.18x^{0.47}$ (1.04－3.09)	$0.53x^{-0.27}$(0.97－1.79)	$2.46x^{0.22}$ (0.87－1.47)
Zn^{2+}	$48.18x^{1.11}$ (0.29－3.72)	$6.08x^{0.31}$ (1.43－2.95)	$0.03x^{-0.92}$(0.37－3.08)	$0.01x^{-1.72}$ (0.29－15.47)
Cu^{2+}	$1.25x^{-0.03}$ (1.34－1.44)	$2.28x^{0.11}$ (1.34－1.75)	$0.51x^{-0.16}$(0.74－1.06)	$0.93x^{-0.01}$ (0.96－0.99)
Ni^{2+}	$17.07x^{0.52}$ (1.55－5.14)	$1.91x^{0.06}$ (1.45－1.67)	$0.71x^{0.25}$(0.23－0.40)	$0.08x^{-0.21}$ (0.13－0.21)
Co^{2+}	$2.21x^{-0.15}$ (3.13－4.42)	$5.36x^{0.47}$ (0.61－1.81)	$0.18x^{-0.81}$(1.15－7.47)	$0.43x^{-0.19}$ (0.66－1.03)
Fe^{2+}	$2.49x^{0.15}$ (1.24－1.76)	$2.77x^{0.43}$ (0.38－1.02)	$0.98x^{-0.49}$ (2.99－9.14)	$1.09x^{-0.20}$ (1.74－2.79)
Mn^{2+}	$4.47x^{0.18}$ (1.92－2.93)	$1.30x^{0.06}$ (0.99－1.14)	$1.59x^{0.03}$ (1.39－1.49)	$0.46x^{-0.09}$ (0.58－0.72)

Note: [a] Domain of functional value; Live. live algal cell; dead. dead algal cell; P. *C. pyrenoidosa*; R. *C. reinhardtii*.

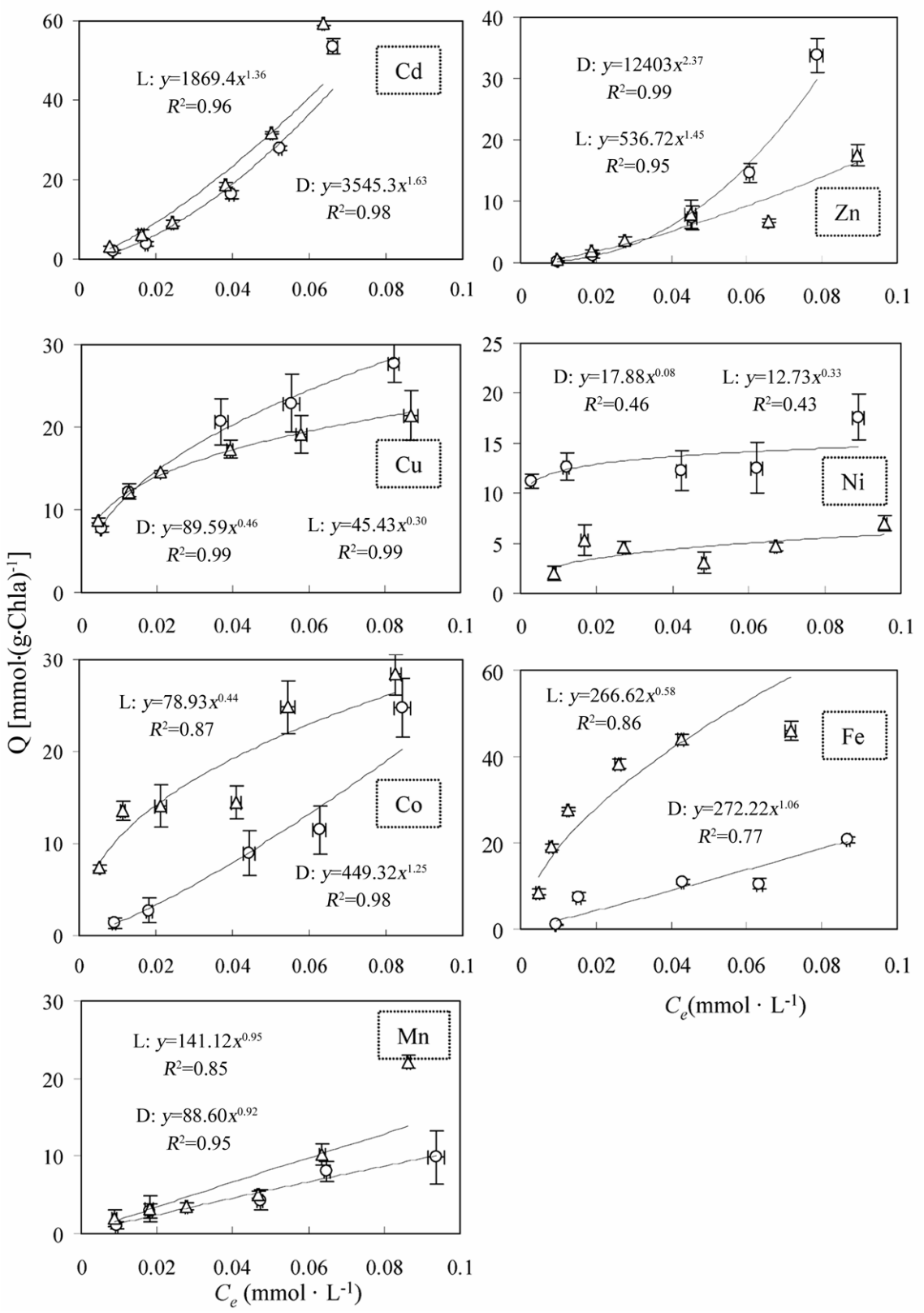

Fig. 1. Biosorption of trace metals from aqueous multi-metal solutions by *C. pyrenoidosa.* Dots stand for dead cells and triangles stand for live cells. D. dead algal cell; L. live algal cell.

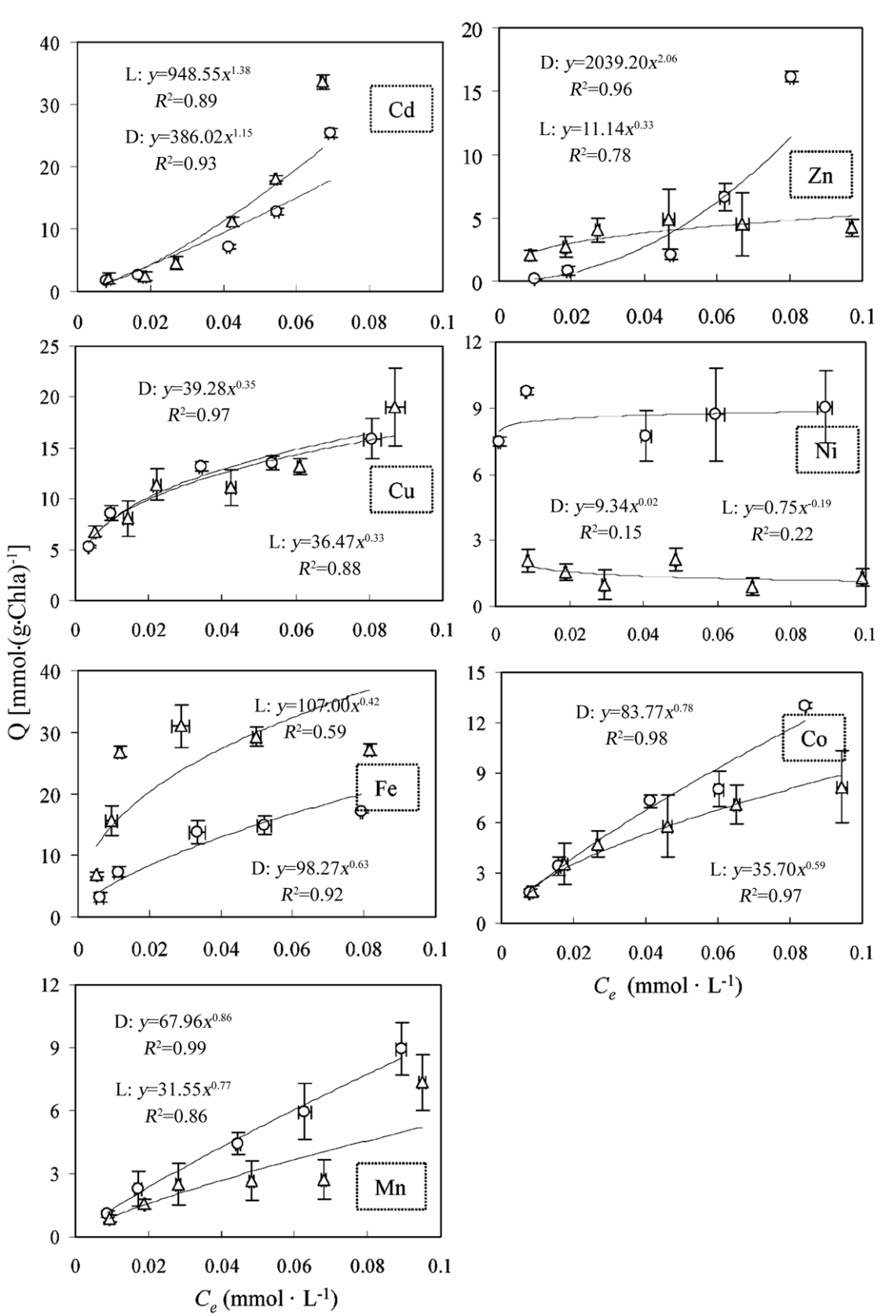

Fig. 2. Biosorption of trace metals from aqueous multi-metal solutions by *C. reinhardtii*. Dots stand for dead cells and triangles stand for live cells. D. dead algal cell; L. live algal cell.

Different trace metals showed different behaviors in the process of biosorption by microalgae. Generally, heavier metals such as Cd^{2+}, Zn^{2+}, and Cu^{2+} were easier to be adsorbed by dead cells than lighter metals such as Mn^{2+} and Fe^{2+}. Biosorption of Mn^{2+} and Fe^{2+} by live and dead cells varied much more than that of other trace metals at the different trace metal concentrations.

4 Discussion

Biosorptions of trace metals are different because of different algal species, mainly due to species-specific characteristics of the components and structures of algal cell walls. The components of cell walls of *C. reinhardtii* and *C. pyrenoidosa* are different. For example, cell wall of *Chlorella* contains 2%–16% protein (Blumriesinger et al., 1983) and that of *Chlamydomonas* at least 32% glycoprotein complex (Miller et al., 1974). Cell walls of microalgae are also different in structure. The cell wall of *C. reinhardtii* appears to be lack in a microfibrillar component and that of *C. pyrenoidosa* contains microfibrillar components with a reticulate pattern (Dawes, 1966). Therefore, a series of processes such as physicochemical sorption, ion exchange, complexation, chelation, and microprecipitation that occur on the cell walls of these microalgae differ from each other, and this finally resulted in their different adsorbabilities. Moreover, the cell size of *C. reinhardtii* is triple the cell size of *C. pyrenoidosa* according to light microscope observation, indicating that the latter has a larger specific surface area than the former. And this may lead to the absorption of more trace metals by *C. pyrenoidosa* than by *C. reinhardtii* (Fig. 3).

Live cells possess a more complex biosorption mechanism than dead ones due to their additional cell-mediated intracellular accumulation, inducing different uptakes of the same trace metal. Boiling algal cells can destroy the protein related to trace metal metabolism and thus influence the absorption of essential trace metals such as Fe^{2+}. This may be the reason why live cells could absorb much more Fe than dead ones (Fig. 3). As for almost all studied trace metals, the ratio of Q_{live} to Q_{dead} decreased with increasing trace metal concentrations (Table 1), suggesting that, compared to dead ones, live cells showed smaller changes in the quantity of bioadsorption. This may be attributed to live cells possessing detoxification mechanism (Zhou Wenbin and Qiu Baosheng, 2004) that can decrease the uptake of trace metals at high concentrations.

Our study also demonstrated that the same biosorbents have different appetites towards different trace metals in the aqueous multi-metal solutions (Fig. 3). This may be ascribed to distinct atomic weight and ion density of each kind of trace metals, considering that the Van Der Waals force and chemical bond are the main driving forces for physical and chemical adsorptions of trace metals by cell walls. We calculated the ion density of each bivalent trace metal with its atomic weight being listed in Table 2. The sequence of biosorption ability on trace metals by *C. pyrenoidosa* and *C. reinhardtii* is also listed (Table 3). As a result, the order of adsorbability on Cd^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}, and Mn^{2+} by dead cells is consistent with the order of atomic weights of these trace metals as the dead cells of those two microalgae prefer heavier trace metals to lighter ones. However, for live cells, the sequence of adsorbability for Cd^{2+}, Fe^{2+}, Co^{2+}, and Zn^{2+} (Ni^{2+}) is opposite to the sequence of ion density of these trace metals as the cell mechanism involves the uptake of these trace metals and trace metal transporter on cell surface prefers smaller ion density due to less energy consumption in the process of transportation. It is clear that competition among trace metals complicates the biosorption by biomaterial.

5 Conclusions

Biosorptions of Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} from aqueous multi-metal solutions by green microalgae *C. reinhardtii* and *C. pyrenoidosa* are different due to different microalgal and trace metal species. This species-specific characteristic may be ascribed to the distinct components and structures of algal cell walls and the different physicochemical characteristics of each kind of trace metals. Live microalgal cells exhibited a more complex sorption process than dead ones because of the involvement of cell assimilation. *C. pyrenoidosa* can be a good biomaterial for biosorption of Cd^{2+}.

Table 2 Related physical properties of each kind of trace metals in this study

	Mn^{2+}	Fe^{2+}	Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}	Cd^{2+}
A	54.94	55.85	58.93	58.71	63.54	65.37	112.4
R (Å)	0.80	0.76	0.74	0.72	0.69	0.74	0.97
r	25.63	30.39	34.74	37.57	46.20	38.53	29.42

Note: A. Atomic weight; R. ion radius; r. ion density; $r=3A/(4\pi R^3)$.

Table 3 Sequence of biosorption ability on trace metals by *C. pyrenoidosa* and *C. reinhardtii*

	C. pyrenoidosa	*C. reinhardtii*
Dead cells	Cd>Zn≥Cu≥Co≥Fe≥Ni>Mn	Cd≥Fe≥Zn≥Cu≥Co≥Ni>Mn
Live cells	Cd≥Fe>Co≥Cu>Mn≥Zn≥Ni	Cd≥Fe≥Cu>Co >Mn≥Zn>Ni

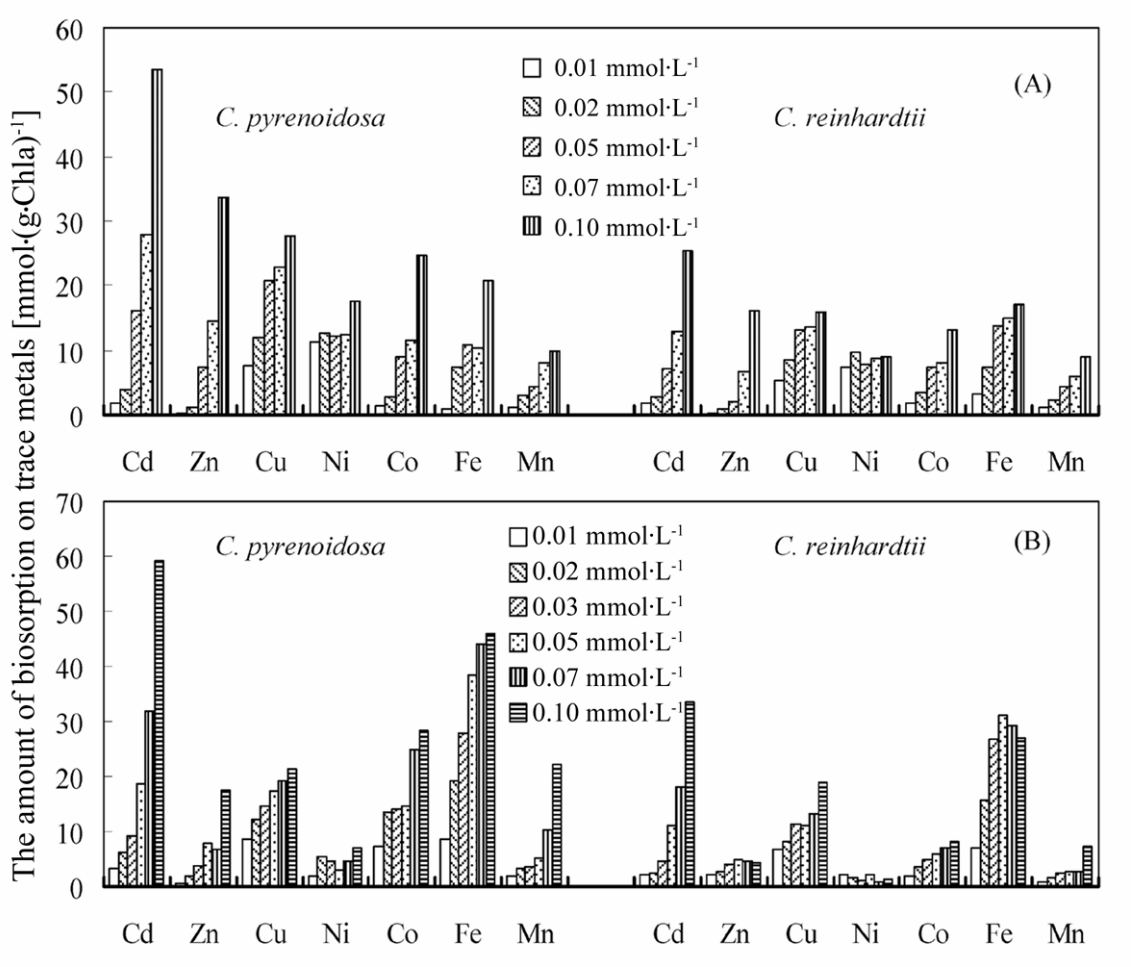

Fig. 3. Biosorption of trace metals by dead (A) and live (B) algal cells at different metal concentrations.

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