



Effect of sorbed and desorbed Zn(II) on the growth of a green alga (*Chlorella pyrenoidosa*)

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Abstract

Toxic effect of Zn(II) on a green alga (*Chlorella pyrenoidosa*) in the presence of sepiolite and kaolinite was investigated. The Zn-free clays were found to have a negative impact on the growth of *C. pyrenoidosa* in comparison with control samples (without adding any clay or Zn(II)). When Zn(II) was added, the algae in the presence of clays could be better survived than the control samples, which was actually caused by a decrease in Zn(II) concentration in the solution owing to the adsorption of Zn(II) on the clays. When the solution system was diluted, the growth of algae could be further inhibited as compared to that in a system which had the same initial Zn(II) concentration as in the diluted system. This in fact resulted from desorption of Zn(II) from the zinc-contaminated clays, although the effect varied according to the different desorption capabilities of sepiolite and kaolinite. Therefore the adsorption and desorption processes of Zn(II) played an important part in its toxicity, and adsorption and desorption of pollutants on soils/sediments should be well considered in natural eco-environmental systems before their risk of toxicity to aquatic organisms was assessed objectively.

Key words: *Chlorella pyrenoidosa*; clays; adsorption and desorption; toxicity

Introduction

In recent years various pollutants, heavy metals in particular, have entered into aquatic ecosystem, adsorbed to the surfaces of suspended solids/sediments and presented many different environmental behaviors (Madoni and Romeo 2006; Honeyman and Santschi, 1988; Karickhoff, 1984). Suspended solids were noted to be able to decrease toxicity of many pollutants for aquatic organisms by decreasing the free concentration of the molecules or ions (Kördel *et al.*, 1997), and dilution was also thought to be a way to decrease the concentration of pollutants in aquatic system. If an aquatic system underwent a dilution process, whether the toxicity of pollutants could be decreased accordingly remained actually unpredictable, as desorption might occur simultaneously during the dilution process. Furthermore, the desorption of pollutants from particles was directly related to adsorption irreversibility, and hence affected their bioavailability (Pan *et al.*, 2002, 2004; Comans and Vandijk, 1988). Therefore adsorption and desorption (irreversibility) of pollutants on particles should be investigated beforehand to fully understand their toxicity in natural eco-environmental systems.

In order to simulate the adsorption and desorption

process occurred in natural eco-environmental systems, two clay minerals, sepiolite and kaolinite, were selected to be contaminated artificially with Zn(II) in a culture medium. An alga *Chlorella pyrenoidosa*, a commonly found freshwater green algal species in China, was chosen as a representative of primary producers (Guschina and Harwood, 2006) to study the toxicity of Zn(II) in the presence of clays. Fewer data were available so far to demonstrate that pollutants (e.g. heavy metals) adsorbed to inorganic particles, such as clay, sand, or silt were bioavailable for aquatic organisms, and it was suggested that contaminated particles should be considered as a separate compartment in risk evaluation of pollutants (Van den Belt *et al.*, 2000; Weltens *et al.*, 2000). Therefore the effect of adsorption and desorption of zinc-contaminated clays on the growth of *C. pyrenoidosa* would be focused on in this study.

1 Materials and methods

1.1 Chemicals, alga and culture medium

Analytical grade of Zn(II) chloride was used in the experiments. *C. pyrenoidosa* was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. Two clay samples, sepiolite ($\text{Mg}_8(\text{H}_2\text{O})_4[\text{Si}_6\text{O}_{15}](\text{OH})_4 \cdot 8\text{H}_2\text{O}$, 2–2.5 g/cm³) and kaolinite ($\text{Al}_4[\text{Si}_4\text{O}_{10}](\text{OH})_8$, 2.6–2.63 g/cm³), were used as adsorbents, whose average particle

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sizes were 22.0 and 7.4 μm respectively (Pan *et al.*, 2006). The culture medium (0.200 g of $(\text{NH}_4)_2\text{SO}_4$, 0.030 g of superphosphate, 0.080 g of MgSO_4 , 0.100 g of NaHCO_3 , 0.025 g of KCl , 0.15 ml of 1% (w/v) $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution and 1000 ml of distilled water) and culture condition for *C. pyrenoidosa*, and the analytical procedures were the same as those previously described (Yan and Pan, 2002). All other chemicals used in this study were analytical grade.

1.2 Methods

1.2.1 Adsorption and desorption experiments

Mix Zn(II) solutions with the algal culture medium and clay samples (0.0125 g of sepiolite or kaolinite), and make the final Zn(II) concentrations at 0, 0.5, 1, 2, 4 and 6 mg/L and a clay concentration of 500 mg/L in a total volume of 25 ml. The pH was adjusted to 7.0 in all cases with NaOH or HCl and the resulting mixture was then shaken continuously for 24 h at 200 r/min and at an ambient temperature of $25 \pm 2^\circ\text{C}$. During this period, the pH of the test system was adjusted in several intervals. At the end of the reaction, the solution was filtered and analyzed for zinc using flame atomic absorption spectrometry.

Desorption experiments were performed after 24 h of adsorption. The mixture after the above adsorption reaction was diluted to $2 \times$ using algal culture medium so that the initial Zn(II) concentration of the desorption system was decreased to half of that of adsorption system. The desorption mixture was allowed to equilibrate for an additional 24 h, after all the other operations as in the adsorption experiment. The concentration of Zn(II) in the desorption solution was then determined.

1.2.2 Toxicity test and determination of chlorophyll-*a*

After the adsorption and desorption processes described above, *C. pyrenoidosa* was inoculated into the two systems and incubated for 96 h. The cells of *C. pyrenoidosa* were filtrated onto 0.22 μm cellulose acetate filters afterwards and dissolved completely with 90% acetone solution and then measured for optical density at the wavelength of 665 nm ($A_{665 \text{ nm}}$). The chlorophyll-*a* concentration was calculated by chlorophyll-*a* (mg/L) = $13.4 \times A_{665 \text{ nm}}$ (Taras *et al.*, 1971). The controls without clay were processed in the same manner.

2 Results and discussion

2.1 Adsorption and desorption of Zn(II) on clays

The adsorption and desorption of Zn(II) on sepiolite and kaolinite and the desorption efficiencies are presented in Fig. 1. It was noted from the adsorption isotherms (Fig. 1a) that the adsorption of Zn(II) on the two clays increased at different rates with the equilibrium concentration (C_{eq}), and sepiolite had much higher (more than doubled) adsorption capacity than kaolinite. The desorption of Zn(II) was found to be increasing with C_{eq} as well, which could be quantitatively described as:

$$R_d(\%) = ((q_{\text{ad}} - q_{\text{de}}) / q_{\text{ad}}) \times 100\% \quad (1)$$

where, R_d is Zn(II) desorption efficiency, q_{ad} is the

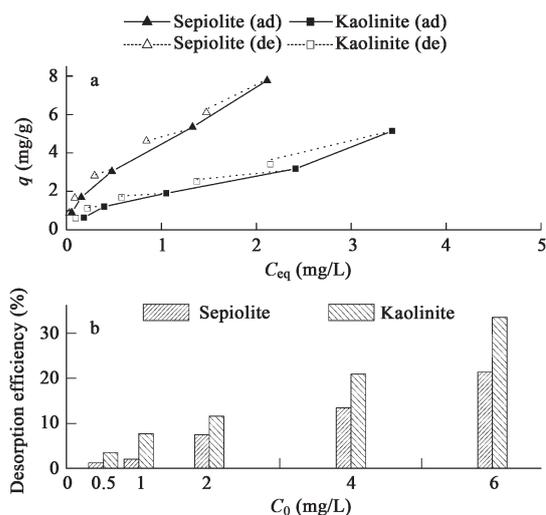


Fig. 1 Adsorption and desorption of Zn(II) on sepiolite and kaolinite. (a) isotherms of zinc adsorption and desorption from sepiolite and kaolinite; (b) desorption efficiency (%) of zinc versus initial zinc concentrations (C_0).

quantity of adsorbed Zn(II), and q_{de} is the quantity of retained Zn(II) after desorption.

It was noticed that the desorption efficiency of Zn(II) from sepiolite was smaller than that of kaolinite at all Zn(II) initial concentrations (Fig. 1b). It indicated that zinc-sepiolite system showed higher adsorption capacity and lower desorption efficiency than zinc-kaolinite system, and adsorbed Zn(II) on the clay minerals could be desorbed as the system was diluted, depending on the type of minerals. As the desorption data points were close to the adsorption isotherms (Fig. 1a), these adsorption-desorptions were rather reversible. All of the above demonstrated that clays varied in adsorption and desorption properties, and the toxicity (bioavailability) of pollutants (heavy metals) was to be determined by the interplay of the two.

2.2 Effect of zinc-contaminated clays

Chlorophyll-*a* was obtained after 96 h exposure of *C. pyrenoidosa* in a series of experimental systems, following the adsorption and adsorption-desorption stages mentioned above. Fig. 2 shows a plot of chlorophyll-*a* against C_0 of Zn(II) in two clay systems of adsorption.

Firstly, when $C_0 = 0$, there was no Zn(II) in the solution and the drop in chlorophyll-*a* was solely the effect of clays on the algal growth. It was interesting to find that there was no obvious difference for the two uncontaminated clay-culture systems under the experimental conditions. This might be attributed to the almost equivalent effects of the two clays of screening and flocculating related to their physical properties (Sengco *et al.*, 2005), which should remain the same throughout all the following experiments.

It was observed that chlorophyll-*a* decreased with increasing C_0 of Zn(II) in the two clays systems. In zinc-contaminated sepiolite system, the values of chlorophyll-*a* were overall higher than those in kaolinite system and the controls with the same initial Zn(II) concentrations, showing less toxicity after 96 h growth period. This was obviously corresponding to the relatively lower Zn(II) concentrations in the sepiolite system due to its higher

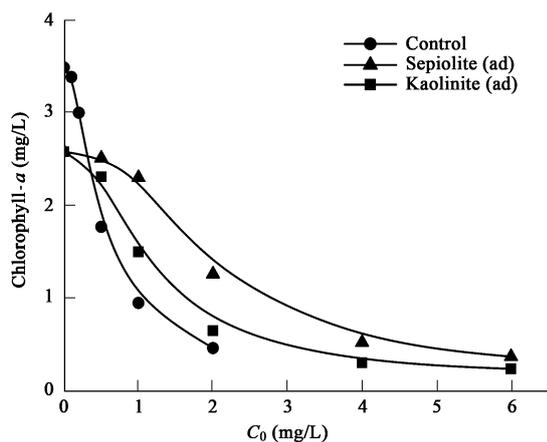


Fig. 2 Effect of initial Zn(II) concentration (C_0) on the growth of the algae in clay-culture medium after 96 h in adsorption stage. The effect of clay alone was shown at $C_0 = 0$.

adsorption capacity of Zn(II). The adsorption of Zn(II) on clays was so great that the zinc-decreasing effect surpassed the physical screening and flocculating effect before the C_0 reached 0.5 mg/L.

After the zinc-contaminated clay systems were performed adsorption-desorption process, these systems were used as mediums to culture the algae, and the effects are shown in Figs.3 and 4 for sepiolite and kaolinite respectively. It could be observed clearly in the zinc-contaminated sepiolite system that the chlorophyll-*a* concentration decreased after dilution process (Fig.3a), and a similar phenomenon was also shown in the zinc-contaminated kaolinite system (Fig.4a). Combining the two plots, it could be seen that chlorophyll-*a* in the sepiolite system was still higher than that of the kaolinite system after dilution.

It is interesting to note both in Fig.3a and Fig.4a that the growth of algae was further inhibited when the systems were diluted, and the inhibition increased with increasing initial Zn(II) concentration. In order to evaluate quantitatively the contribution of previously bound zinc to the toxicity, an indicator for the effect of desorption (E_d) could be introduced as:

$$E_d(\%) = (q_{ad(Chl-a)} - q_{ad-de(Chl-a)}) / q_{ad(Chl-a)} \times 100\% \quad (2)$$

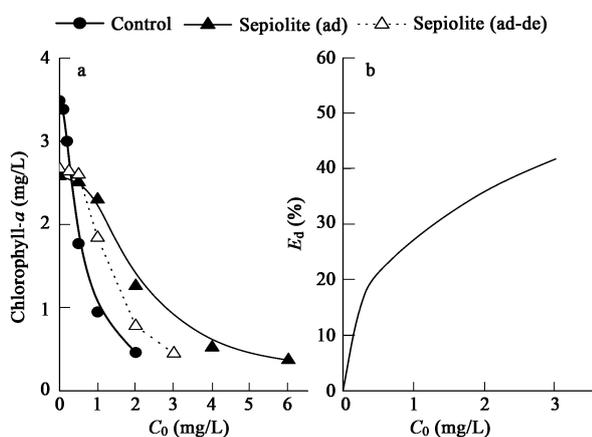


Fig. 3 Effect of Zn(II) on the growth of algae in sepiolite-culture medium. (a) the systems before and after dilution, and (b) the effect of desorption (E_d (%)) on the algae.

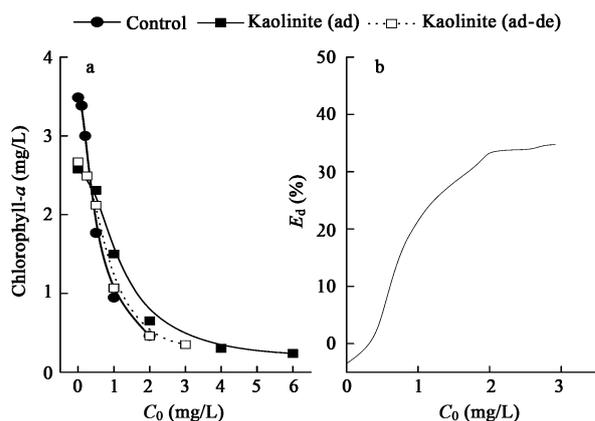


Fig. 4 Effect of Zn(II) on the growth of algae in kaolinite-culture medium. (a) the systems before and after dilution, and (b) the effect of desorption (E_d (%)) on the algae.

where, $q_{ad(Chl-a)}$ is the chlorophyll-*a* of algae after adsorption of Zn(II); and $q_{ad-de(Chl-a)}$ is the chlorophyll-*a* of algae after the treatment of Zn(II) adsorption and desorption.

Figures 3b and 4b present the changes in the value of E_d at different initial zinc concentrations (C_0). If there were no desorption of Zn(II) occurred in the dilution process (completely irreversible), it would have been difficult to understand the increase in inhibition with C_0 , because the dilution would decrease the particle concentration in half and hence reduce the effect of screening and flocculating and make the algae grow better. The only possible explanation for this was that the zinc bound to clays desorbed from the particle's surface and exerted a significant contribution to toxicity to algae, and the extent (percentage) of contribution increased with increasing total amount of zinc in the systems, well in agreement with the trend in zinc desorption efficiency (Fig.1b). This phenomenon strongly indicated that desorption and irreversibility were essential in natural dilution processes, and they varied with initial concentration of pollutants and physicochemical properties of particles.

Particles may serve as major storage reservoirs for pollutants, since adsorption of pollutants on suspended solids could decrease toxicity (Rodgers *et al.*, 1987). However, in the present case, results showed that algae suffered low toxicity in zinc-contaminated clay systems before dilution but when the systems were diluted, toxicity increased instead of decreased remarkably. The phenomenon indicated that contaminated-clays and sediments might also be the "source" of pollutants (heavy metal ions) and release the pollutants into aquatic system under the changes in physicochemical environment. The bound or release of heavy metals on particles in aquatic system was related to their irreversibility i.e. the microscopic states of adsorbed species as proposed by the MEA theory (Pan and Liss, 1998a, b), and this could affect bioavailability and hence toxicity of the heavy metals in natural waters (Pan *et al.*, 2002, 2004; Comans and Vandijk, 1988). Although the clays used in this study had not presented fundamental difference in adsorption irreversibility or size effect, the differences in all other natural minerals (soils/sediments) and artificial materials (nanoparticles) could not be ne-

glected, and the toxic effects of contaminated particles and the role of the fraction of adsorbed pollutants on its toxicity had yet to be investigated in detail.

3 Conclusions

These experiments indicated that uncontaminated clays themselves could inhibit the growth of algae, and particles could exert a dual influence on pollutants. One was to decrease the toxicity due to adsorption and the other was to increase the toxicity due to desorption. In aquatic systems the adsorption and desorption, and the irreversibility of pollutants on suspended particles should be taken into account seriously in risk evaluation of pollutants in natural waters.

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