Synergetic Effect of Polynuclear Hydroxyl-aluminium and Coexisting Zinc on the Survival of Aquatic Diatom

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Abstract: Attached algae are one of the main constituents in a river ecosystem since they are the primary producer and serve as food for aquatic insects and fishes. Therefore, their preservation is important for river environment. In this study, the effect of metals on the decay of Nizschia palea, diatom, was investigated by batch experiments. Polynuclear hydroxyl-Al (Al-b) at the concentration of 0.3 mg/l and Zn higher than 0.2 mg/L accelerated the decay of N. palea, while Ca higher than 10 mg/L meditated the toxicity of Al-b. Zn and Al-b had an additive or synergetic action on the decay of N. palea at the concentrations of higher than 0.1 mg/L and 0.03 mg/L, respectively. This suggests that combined effect of metals should be considered for river environments and establishing water quality standard for aquatic biota.

Keywords: Nizschia palea, polynuclear hydroxyl-aluminium, synergetic effect, toxicity, calcium, zinc

INTRODUCTION

Nowadays, importance of conservation of aquatic biota has been noted. In Japan zinc was selected for the water quality standard to preserve aquatic biota in fresh waters and set as 0.03 mg/L in 1994. In a river ecosystem attached algae are the primary producer and serve as food source for aquatic insects and fishes. Therefore, their preservation is essential for aquatic environments.

In this study we focused on the inhibitory effect of aluminium and coexisting metals on attached alga. Aluminium is one of the main constituents in the earth crest and soils contain Al with a high content. Al is easily released from soils to aquatic environments by acidification and is discharged from active or abandoned mines. The form of Al present in an aquatic environment is categorized into three: mononuclear Al (Al-a) such as Al\(^{3+}\) and AlOH\(^{2+}\), polynuclear hydroxyl-Al (Al-b) such as Al\(_6\)(OH)\(_{13}\)\(^{3+}\) and Al\(_{13}\)O\(_4\)(OH)\(_{24}\)\(^{7+}\), and insoluble Al (Al-c) such as Al(OH)\(_3\). Al-a is a predominant species at pH values less than 4, and Al-b followed by Al-c dominates as pH increases (Yan et al. 2007). It has been reported that Al-b had the highest toxicity to plants (Parker et al. 1989; Comin et al. 1999), and its toxicity was caused by adsorption of Al to cell walls and plasma membranes and inhibited the uptake of Ca and Mg and the release of hydrogen ion (Barceló et al. 2002). Gensemer et al. (1999) reviewed the aluminium toxicity to freshwater algae and pointed out that its toxicity is the highest at pH around 6 and the speciation of Al is necessary for estimating Al toxicity. It has been reported that elongation of cell of alga Chara coralline was inhibited within 2 hours and stopped completely within 6 hours by 0.1 M of Al at pH 4.4 (Reid et al. 1995), photosynthetic efficiency of
green flagellate (*Euglena gracilis*) was adversely affected at Al concentrations from 0.5 to 15 mg/L (Danilov *et al.* 2002), and Al extracted from the sludge produced in water treatment plants was toxic to green alga (*Selenastrum capricornutum*) when water hardness was less than 35 mg/L as CaCO$_3$ (George *et al.* 1995). However, these results did not consider the form of Al in water although the toxicity of Al depends on its form. Furthermore, combined effect of Al species and other metals should be studied because existence of only one metal is not realistic in aquatic environments and information on toxicity of such metals to diatom was lacking.

The objectives of this study are to make clear the toxicity of Al species to diatom *Nitzschia palea*, and combined effect of Al species and Ca or Zn on the decay of *N. palea* in batch cultures with pH 6.

**MATERIALS AND METHODS**

The reagents used in this study are all of analytical grade. The solution containing a high (Solution II) or low content (Solution I) of polynuclear hydroxyl-Al was prepared by the following procedure. The stock solution containing 400mg/L of Al was prepared by dissolving AlCl$_3$·6H$_2$O (Kanto Chemicals) with a dilute HCl solution. The molar ratio of Al and HCl was 0.5 and the pH was 1.4. The Solution I was prepared by directory diluting the stock solution with ultra pure water and adjusting the pH to 6 with NaOH. The Solution II was prepared by increasing the pH of the 1 L stock solution with 10 M NaOH to 4.5 and diluting it to 2 L with a HCL solution (pH 4.5) to promote hydrolysis. Then, the solution was diluted to adjust the Al concentration with ultra pure water and the pH to 6 with a dilute NaOH solution. The nominal concentrations of total Al (Al$_T$) for testing the effect of Al species were 0.5, 1.0 and 5 mg/L in both the Solutions I and II. The concentrations of three Al fractions and Al$_T$ in the Solution I and II were determined by ferron colorimetric method (Parker *et al.* 1992) and using ICP-MS (Yokogawa Analytical Systems HP-4500).

The stock solutions of zinc and calcium were prepared by dissolving ZnCl$_2$ and CaCl$_2$ (Kanto Chemicals) with ultra pure water. *Nitzschia palea* (NIES-487, diatom) obtained from the NIES (National Institute for Environmental Studies) Collection, Japan was used in this study and this was cultured in the CSi medium (National Institute for Environmental Studies JAPAN, 2004) with a light-dark cycle of 12 hours at 25°C and a photon flux density of 53 μmol/m$^2$/s in the laboratory. At the end of logarithmic growth phase, the diatom was collected by centrifuging the medium at 10,000 rpm for ten minutes and then washed three times with deionized water by repeating centrifugation to remove constituents in the medium. The experiments were conducted by inoculating *N. palea* in 3 L erlenmeyer flasks containing single or two kinds of metals with various concentrations and cultured with a photon flux density of 53 μmol/m$^2$/s at 25°C and pH 6.0 by adding buffer agent of 2-Morpholinoethanesulfonic acid (MES, 1.68 mM). The flasks were stirred several times a day. The sampling was conducted at 0, 6, 12, 24, 48 and 72 hours from the start of experiments and the concentrations of chlorophyll $a$ were analyzed (three times in each sample). The plots in the following figures are the average values. In this study nutrients were not added to the flasks to avoid the formation of aluminium phosphate and aluminium complexes.
RESULTS AND DISCUSSION

Effect of Al species on the decay of Nitzschia palea

Figure 1 shows the concentrations of Al species in this experiment. Each length of the bar in this figure indicates the concentration of Al species. It is seen that the Solution II contained higher content of Al-b compared with that of the Solution I at the same total Al (Al_T) concentrations.

![Figure 1: Content of Al species in Solution I and II](image)

Figure 2 shows the variations of chlorophyll a concentrations in the blank (Al_T =0mg/L), Solution I and Solution II. The concentrations of chlorophyll a decreased even in the blank solution since the nutrients were not added in this experiment and the profile of chlorophyll a with time in the blank and Solution I at Al_T of 0.5 and 1.0 mg/L was almost same, indicating that Al-a less than 0.2 mg/L and Al-c less than 0.8 mg/L (Figure1(b)) did not affect the decay of N. palea. It is worth noting that the rates of decrease in chlorophyll a concentrations in the Solution II containing 0.5 and 1.0 mg/L of Al_T were higher than those in the Solution I and the rates of decrease were almost same in 5 mg/L of Al_T. This indicates that Al-b of 0.3 mg/L (Figure 1(a)) accelerated the decay of N. palea and both Al-b and Al-c also accelerated at 5 mg/L of Al_T. Parent and Cambell (1994) reported that Al-b of 0.2 mg/L with pH 6.0 inhibited the growth rate of Chlorella pyrenoidosa by 90% in 4 days compared with that of containing 0.19 mg Al-a/L. It is interesting that these values are close although the taxonomic group of algae studied differs. At present, only limited researches have been conducted on the inhibitory effect of Al-b on algae. In this experiment N. palea was suddenly exposed to Al. Since a good acclimatization capacity of Euglena gracillis to Al has been reported (Danilov et al. 2002), further study should be required.

![Figure 2: Variation of chlorophyll a concentration at pH 6](image)
Effect of Zn on the decay of *Nitzschia palea*

The variation of chlorophyll *a* concentrations with time at different Zn concentrations is shown in Figure 3. The vertical axis shows the ratio of chlorophyll *a* concentration at time t (*C*<sub>t</sub>) to initial one (*C*<sub>0</sub>) as *C*<sub>0</sub> ranged from 150 to 220 μg/L. It was found that after 48 hrs from the start of the experiment Zn higher than 0.2 mg/L accelerated the decay of *N. palea*, while Zn less than 0.1 mg/L did not affect. Wilde *et al.* (2006) showed that the minimum detectable effect concentration (MDEC) of Zn for *Chlorella* *sp.* at pH 6.0 was 0.35 mg/l although the toxicity of metal is algal species dependent. Japan has established the water quality standard for the preservation of aquatic biota as 0.03 mg Zn/L. Zinc concentration of 0.1 mg/L not affecting the decay of this diatom supports the validity of this standard, although other alga should be tested.

![Figure 3: Variation of chlorophyll *a* concentrations at various Zn concentrations](image)

Effect of combined Al and Ca on the decay of *Nitzschia palea*

Figure 4 shows the concentrations of Al species in the Solutions I and II and Ca in the Solution II. The nominal Al<sub>T</sub> concentration was 1mg/L and in this experiment Ca was added to the Solution II only as the effect of Al-b on the decay was significant. It was seen that the concentrations of Al-b in the Solution II were about 0.8 mg/L, which accelerated the decay of *N. palea* as shown previously, and the addition of Ca to the Solution II did not change the content of Al species.

![Figure 4: Concentrations of Al species and Ca](image)

The variations of chlorophyll *a* concentrations with time in the blank (both Al and Ca not added), Solutions I and II (Al added), and Solution II (both Al and Ca added) were shown in Figure 5.
For example, Ca:10 in this figure means the concentration of Ca was 10mg/L. Comparing the results of the Solution I, Solution II and Ca:200 with that of the blank, the Solution I and the addition of 200 mg/L of Ca did not affect the decay of *N. palea*, while the Solution II did significantly. Furthermore, the addition of Ca to the Solution II mitigated the rate of decay, indicating the antagonistic action of Ca and Al-b. Alleviative action of hardness such as Ca and Mg to the inhibition of copper (Heijerick *et al.* 2005), uranium (Charles *et al.* 2002) on green alga and nickel on cladocerans (Nele *et al.* 2007) has been reported, and explained that hardness components compete the binding sites of cell membrane with other metals although contradictory result also reported (Makich *et al.* 2005). However, this action could not serve effectively since the concentration of Ca in fresh water environment is around 10 mg/L in Japan (Hanya *et al.* 1995).

**Figure 5:** Effect of Ca addition on the decay of *Nitzschia palea*

**Effect of combined Al and Zn on the decay of *Nitzschia palea***

Figure 6 shows the concentrations of Al species. In this figure Zn means that zinc of 0.03 mg/L was added to the Solution I or Solution II and the numerical values show AlT concentrations. As shown in the previous section, Zn of 0.03 mg/L did not inhibit the survival of *N. palea*. It is seen that the addition of Zn did not change the content of Al species and the Solution II was composed of mainly Al-b and Al-c as with the results of previous experiments.

**Figure 6:** Experimental conditions
The experiment to that of initial one (C concentration of Al-b and the ratio of chlorophyll and Al-b itself inhibited the survival of N. palea as shown previously. On the other hand, in the Solution II containing a relatively high content of Al-b, the rates of decay under the condition of combined Al\textsubscript{T} of 0.2, 0.5 and 1.0 mg/L and Zn of 0.03 mg/L were higher than those of no addition of Zn, whereas those of Al\textsubscript{T} of 2.5 mg/L were almost same in the addition and no addition of Zn due to the inhibition of Al-b since the concentration of Al-b was more than 1 mg/L (Figure 6). These results suggest that Al-b and Zn had an additive or synergetic action at the low concentrations and Al-b itself inhibited the survival of N. palea at high concentrations.

In order to make clear the combined effect of Al-b and Zn, the relationship between the concentration of Al-b and the ratio of chlorophyll \(a\) concentration at 48 hours (\(C_{A0}\)) from the start of the experiment to that of initial one (\(C_0\)) is depicted in Figure 8. The ratios decreased with increasing the concentrations of Al-b due to inhibitory effect of Al-b. Comparing the results with addition of Zn with those of no addition, it is obvious that the ratios with addition of Zn were smaller than those with no addition of Zn and the difference of the ratio was the largest at about 0.1 mg/L of Al-b (Al\textsubscript{T}=0.5 mg/L). This implies that water quality standard of metals for aquatic biota must take into account combined effect of metals because various metals with various concentrations are present in rivers.

**Figure 7:** Combined effect of Al and Zn on the decay of *Nitzschia palea*
CONCLUSIONS
The effect of Al, Zn, coexisting Al and Ca, and coexisting Al and Zn on the decay of *Nitzschia palea* was studied at various metal concentrations in batch experiments. The experiments were conducted by not adding nutrients to the medium to avoid the formation of Al complexes and Al precipitates. Polynuclear Hydroxyl-aluminium (Al-b) of 0.3 mg/L accelerated the decay of *N. palea* and Zn less than 0.2 mg/L did not affect the decay of *N. palea*. Ca at the concentrations of higher than 10 mg/L mitigated the toxicity of Al-b. When both Al-b and Zn were present, additive or synergetic action were observed at the concentrations higher than 0.1 mg Al-b/L and 0.03 mg Zn/L. These results suggest that water quality standard of metals for aquatic biota must consider combined effect of metals.

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REFERENCES


**Figure 8**: Relationship between Al-b and C_{48}/C_{0}.


